INSTRUCTION MANUAL

MODEL L-7485 FLUORESCENCE DETECTOR

Read and keep this manual

- Read safety instructions carefully and understand them before starting your operation.
- Keep this manual at hand for reference.

HITACHI

INSTRUCTION MANUAL

FOR

MODEL L-7485 FLUORESCENCE DETECTOR

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INSTRUCTION MANUAL FOR MODEL L-7485 FLUORESCENCE DETECTOR

PREFACE

The Model L-7485 fluorescence detector is designed for fluorophotometric analysis of sample components in the liquid chromatograph system.
This instruction manual has been prepared for users of Model L-7485 fluorescence detector. It describes the operation, checkup and maintenance procedures for the L-7485 fluorescence detector.
The Model L-7485 is intended for use by persons having a basic knowledge of chemical analysis. Remember that improper use of the analytical instruments, chemicals or samples would result not only in wrong analytical data but also in consequences adverse to safety.
Read this instruction manual carefully before attempting operation. For proper use of this instrument, please acquaint yourself with it.

ABOUT THIS MANUAL

As mentioned above, this manual has been prepared for users of the Model L-7485 fluorescence detector and covers the operation, checkup and maintenance procedures for the detector. Prior to operation, be sure to read through the SAFETY SUMMARY at the beginning of the manual. Then proceed to Chapter 1 for the installation. Beginners should then go to Chapters 2, 3 and 4 to understand the outline and fundamental operation of the instrument.

IMPORTANT

Precautions on Electromagnetic Wave Interference

1. Possible Electromagnetic Wave Interference Caused by This Instrument

When this instrument is used in a residential area or an area adjacent thereto, it may cause interference to radio and television reception.
To prevent this, use the specified system connection cables in strict accordance with the instruction manual. The instrument is designed to minimize possible electromagnetic wave interference caused by it provided the specified cables are connected properly. However, there is no guarantee that electromagnetic wave interference will not be caused by the instrument.
If the instrument does cause interference to radio or television reception, which can be determined by turning the instrument off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Change the direction of the radio/TV antenna.
- Increase separation between the instrument and the radio/TV receiver.
- Connect the instrument to an outlet on a circuit different from that to which the radio/TV receiver is connected.

2. Possible Electromagnetic Wave Interference Affecting This Instrument

If this instrument is used near an intense electromagnetic source, interfering noise may be given to the instrument and cause an adverse effect on its performance or functionality.
To prevent this, use the specified system connection cables in strict accordance with the instruction manual. The instrument is designed to minimize possible electromagnetic wave interference affecting it provided the specified cables are connected properly. However, there is no guarantee that electromagnetic wave interference will not occur in this instrument.
If the instrument does incur electromagnetic wave interference, which can be determined by turning on and off possible source(s) of electromagnetic interference nearby, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient the instrument.

- Increase separation between the instrument and possible source(s) of electromagnetic interference.
- Connect the instrument to an outlet on a circuit different from that to which possible source(s) of electromagnetic interference are connected.
- Check that any other device connected with the instrument is not affected by electromagnetic interference.

PRODUCT WARRANTY

The Model L-7485 Fluorescence Detector is warranted to be free from defects in material, workmanship and operation under normal use within the product specifications indicated in this manual for the period stated below.

(1) Scope of Warranty

Liability under this warranty is limited to repair, adjustment or replacement by Hitachi or its approved representative. Any parts which prove to be defective during the warranty period will be repaired, adjusted or replaced without charge. Note that a substitute part may be used for repair, or replacement with an equivalent product may be made instead of repair. Also, such system components as a personal computer and printer that are frequently modified for improvement may not be available in original versions at the time of replacement.

The warranty contained herein is for the benefit of and shall be enforceable by the original purchaser of this instrument and is not transferable.

Consumable parts and operating supplies are excluded from this warranty.

(2) Warranty Period

One year from the date of initial installation.

(In case a separate warranty document has been issued, the warranty period indicated therein shall take precedence over the above period.)

(3) Warranty Service

Shall be provided within the business hours specified by Hitachi.

(4) Exclusions From Warranty

Note that this warranty is void in the following cases even within the warranty period.

(a) Failure due to operation at a place not meeting the installation requirements specified by Hitachi.

(b) Failure due to power supply voltage/frequency other than specified by Hitachi or due to power failure.

(c) Corrosion or deterioration of the tubing due to impurities contained in gas, air or cooling water supplied by the user.

(d) Corrosion of the electric circuits or deterioration of the optical elements due to highly corrosive atmospheric gas.
(e) Failure due to use of hardware, software or spare parts other than specified by Hitachi.
(f) Failure due to improper handling or maintenance by the user.
(g) Failure due to maintenance or repair by a service agent not approved or authorized by Hitachi.
(h) When the instrument is disposed of or resold without consulting Hitachi.
(i) Failure attributable to movement or relocation after installation.
(j) Failure due to disassembly, modification or relocation not approved by Hitachi.
(k) Consumables and parts having a limited period of warranty.
(l) Failure due to use of non-warranted parts (parts not described in the instruction manual).
(m) Failure due to acts of God, including fire, earthquake, storm, flood, lightning, social disturbance, riot, crime, insurrection, war (declared or undeclared), radioactive pollution, contamination with harmful substance, etc.
(n) Damage to the instrument, basic software, application software and/or data caused by a computer virus.
(o) Damage to the personal computer used in the instrument, or to basic software, application software and/or data because of momentary voltage drop caused by lightning or power interruption.
(p) Damage to the personal computer, basic software, application software and/or data caused by turning off the PC main power without taking the normal termination procedure.

(5) Limitations on Warranty

HITACHI MAKES NO WARRANTIES, EITHER EXPRESS OR IMPLIED, EXCEPT AS PROVIDED HEREIN, INCLUDING WITHOUT LIMITATION THEREOF, WARRANTIES AS TO MARKETABILITY, MERCHANTABILITY, FOR A PARTICULAR PURPOSE OR USE, OR AGAINST INFRINGEMENT OF ANY PATENT.
IN NO EVENT SHALL HITACHI BE LIABLE FOR ANY DIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY NATURE, OR LOSSES OR EXPENSES RESULTING FROM ANY DEFECTIVE PRODUCT OR THE USE OF ANY PRODUCT.
NO ORAL OR WRITTEN INFORMATION OR ADVICE GIVEN BY HITACHI, ITS DEALERS, DISTRIBUTORS, AGENTS OR EMPLOYEES SHALL CREATE A WARRANTY OR IN ANY WAY INCREASE THE SCOPE OF THIS WARRANTY.

OTHER PRECAUTIONS

(1) Handling of Chemicals and Samples

(a) The user is responsible for following relevant legal standards and regulations in the handling, storage and discarding of chemicals and samples used in analytical operations of this instrument.

(b) Reagents, standard solutions and accuracy-control samples shall be handled, stored and discarded as instructed by the respective suppliers.

(2) Notice on This Instruction Manual

(a) The information contained in this manual is subject to change without notice for product improvement.

(b) This manual is copyrighted by Hitachi with all rights reserved. No part of this manual may be reproduced or transmitted in any form or by any means without the express written permission of Hitachi.

⚠ SAFETY SUMMARY

⚠ General Safety Guidelines

Before using the L-7485 fluorescence detector, carefully read the safety instructions given below.

- Operate the instrument according to the instructions in this manual.
- Installation at delivery of the instrument must be carried out only by personnel who have received training at, and who are authorized by, Hitachi. It should not be installed by the customer.
- Be sure to observe the warnings indicated on the product and in the instruction manual. Failure to do so could result in personal injury or damage to the product.
- The hazard warnings which appear on the warning labels on the product or in the manual have one of the following alert headings consisting of an alert symbol and a signal word DANGER, WARNING or CAUTION.

⚠ **DANGER**	:	Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. (This warning does not apply to this product.)
⚠ **WARNING**	:	Indicates a potentially hazardous situation which, if not avoided, can result in death or serious injury.
⚠ **CAUTION**	:	Indicates a hazardous situation which, if not avoided, will or can result in minor or moderate injury, or serious damage to the product.
⚠	:	The alert symbol shown at left precedes every signal word for hazard warnings, and appears in safety-related descriptions in the manual.
NOTICE	:	Used to present warnings which are not directly related to personal injury hazards, but concern the proper use of the product.

SAFETY SUMMARY

General Safety Guidelines (Continued)

Precautions prior to Use

- Be sure to read the instruction manual carefully and acquaint yourself with the instrument before use.
- Keep the instruction manual nearby so it can be referred to whenever needed.
- Be sure to observe the methods specified in the manual for usage of the instrument.
- Make sure to understand and follow the safety instructions given in the manual.
- Absolutely avoid modifying the instrument, using non-specified parts or removing safety devices as this could be hazardous.
- When using chemicals for analytical operation, be sure to provide proper ventilation of the laboratory room per local requirements. Inadequate ventilation could endanger human health.

The above precautions must be strictly observed. Failure to do so could result not only in incorrect analytical data but also personal injury.

SAFETY SUMMARY

General Safety Guidelines (Continued)

Precautions on Installation, Maintenance and Relocation

- Installation at delivery, maintenance and relocation should be referred to specialists authorized by Hitachi.

- At the time of installation, check in the presence of the customer that all the standard accessories have been supplied. If anything is missing or damaged or if you have any question, contact your nearest service representative. Lack of a standard accessory or accessories may cause the instrument to malfunction and compromise the safety.
  In such case follow the instructions of the installer.

- Do not perform any operation or action other than described in this manual. When in doubt, please contact your local Hitachi sales representative or nearest Hitachi service office.

- Keep in mind that the hazard warnings in this manual or on the product cannot cover every possible case, as it is impossible to predict and evaluate all circumstances beforehand. Be alert and use your common sense.

⚠ SAFETY SUMMARY

⚠ WARNING: Ignition of Flammable Chemicals!

Handling of Flammable Chemicals

- Beware of ignition hazard when using flammable chemicals such as organic solvents.

- Always check the following conditions. If an abnormality is found, stop operation immediately.
  - ◊ Leakage of solvent or waste solution
  - ◊ Leakage of solvent inside the instrument
  - ◊ Inadequate ventilation of the laboratory room

- This instrument is not explosion-proof. During unmanned operation do not use organic solvents having an ignition point below 70 °C.

- When using flammable chemicals, be careful about possible ignition due to static electricity. Particularly when using non-conductive chemicals, employ a conductive vessel made of metal or the like and provide grounding connection correctly.

(section 1-5-3)

⚠ WARNING: Explosion of Vapor from Flammable Chemicals!

Handling of Flammable Chemicals

- If a flammable chemical such as organic solvent leaks from the flow path of the instrument and its vapor concentration exceeds the explosion limit, it may cause spontaneous combustion with dangerously explosive results.

- When using a flammable and readily volatile chemical, be sure to check for leakage from the instrument flow path and ventilate the laboratory room adequately.

(section 1-5-3)

SAFETY SUMMARY

WARNING: Electric Shock in Contact with Inside of Instrument!

Beware of Electric Shock.
Potentially Dangerous Voltages are Present within the Instrument.

- Before removing the instrument cover for replacement or adjustment of internal parts, be sure to turn OFF the power switch and unplug the power cord.

- Before removing the lamp cover for replacement of the light source lamp, be sure to turn OFF the power switch.

- The xenon lamp utilizes a power supply of 30 kV, so there is a danger of serious or fatal injury due to electric shock.
  Before removing the light source cover for lamp replacement, etc., turn off the power and wait at least two (2) hours for the lamp to cool down.

(section 6-7)

WARNING: Electric Shock due to Improper Grounding!

Ground Properly to Prevent Electric Shock Hazard.

- Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.

- This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.

- If a grounded 3-wire outlet is not available, then be sure to provide proper grounding connection.

SAFETY SUMMARY

WARNING: Explosion of Xenon Lamp!

Handling of Xenon Lamp

- The xenon lamp is filled with high-pressure gas (approx. 1 MPa at room temperature, approx. 4 MPa under operating condition). If a strong shock or impact is applied to the lamp or if the surface of quartz glass container is scratched, it may explode and scatter glass pieces, resulting in personal injury.

- When handling the xenon lamp, be sure to wear safety goggles (mask), thick long sleeves, gloves and other proper protective gear.

- Do not touch the quartz glass part of xenon lamp with bare hands. If the xenon lamp is turned on with dust or fingerprints left on the surface of quartz glass part, it may cause contamination burn-in or devitrification, resulting in a decrease in radiant intensity or mechanical strength of glass part. If the quartz glass part of xenon lamp is contaminated with dust or fingerprints, wipe it using the furnished cleaning paper or gauze/absorbent cotton cloth moistened with high-quality alcohol. In this cleaning, be careful not to apply strong impact or shock.

- Be sure to mount the xenon lamp in the specified direction. If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp. Mount the lamp so that the '+'(anode) mark on it will be positioned at the support metal of the lamp holder. If a lamp with its cathode consumed excessively is used continuously, the pressure inside the lamp bulb may become too high, causing explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed significantly.

- If the nut fastening the lamp socket and wiring section is loose, the contact resistance may increase due to poor contact, causing generation of heat and possibly a lamp explosion due to it. The nut must be securely fastened.

SAFETY SUMMARY

WARNING: Explosion of Xenon Lamp! (Continued)

Handling of Xenon Lamp

- Replace the xenon lamp before its cumulative turn-on time exceeds the guaranteed useful lifetime. With the lapse of the guaranteed useful lifetime, the bulb wall darkens due to evaporation of electrode material and adhesion of scattered substances, resulting in a decrease in heat dissipation. This may increase the temperature (pressure) inside the bulb excessively and cause an explosion.

- Before removing the lamp for replacement, etc., turn off power and wait at least two hours for the lamp to cool down. High-pressure gas remains even in a used lamp. Careless handling may result in an explosion. Handle carefully the same as with a new lamp.

- Attach the furnished protective cover to the used lamp as well. Before detaching the lamp, wait until it cools down to room temperature and then replace. Keep the protective cover attached even after replacement. Remember that high-pressure gas remains in the used lamp also. If handled carelessly, it may explode and cause personal injury. Exercise utmost care when handling the used lamp also. (sections 6-1, 6-2)

SAFETY SUMMARY

WARNING: Injury due to Lamp Explosion!

- Explosion of the xenon lamp could cause serious injury. High-pressure gas at about 1 MPa is filled in the lamp at room temperature. Be careful not to apply force to the lamp bulb (glass part) when replacing the lamp. And wear protective goggles and gloves when handling the lamp.

CAUTION: Disposal of Used Lamp

- Wrap the used lamp completely in a cloth (preferably about 3 layers of cloth), then crush the glass part with a hammer or the like, and discard it together with hazardous objects.

CAUTION: Touching Hot Part Could Result in Burns!

- The xenon lamp remains hot for a while even after power-off and can severely burn you if touched. Before adjustment or replacement of the lamp, turn power off and wait at least two hours for the lamp to cool down.

- Upon detaching the light source cover, the xenon lamp is automatically turned off by a safety mechanism.
  Avoid releasing this safety mechanism.

(sections 6-2, 6-7)

CAUTION: Directly Gazing at Illuminated Xenon Lamp Could Cause Eye Damage!

- The xenon lamp radiates intense ultraviolet light when it is on. Do not look at the lamp directly when it is lit. Be sure to wear tinted safety glasses to avoid eye damage.

(sections 6-2, 6-7)

SAFETY SUMMARY

CAUTION: Heavy Instrument!

- This instrument weighs as much as 19 kg. Be careful not to drop it when carrying. Hold the front and rear parts of the instrument firmly when carrying.

(section 1-1)

CAUTION: Fatigue during Long Use!

- Operating the instrument while watching the display in the same posture for long hours will cause fatigue of the eyes and/or body. In consideration of one's health, it is recommended to take a break for 10 to 15 minutes every hour to relax the body.

SAFETY SUMMARY

NOTICES:

Restriction on Use of Reagents

- Fluorocarbon resin and quartz crystal are used in the flow path of this instrument. Never use reagents that would corrode these materials.

Precautions on Use of Corrosive Solvents

- The drain path for carrying leakage solutions is made of polypropylene.

- The materials inside the instrument are susceptible to corrosion by strong acid, strong alkali and organic solvents.

- When using corrosive solvents, make sure that the tubing connections are not loose.

- Using the pump pressure limiter function or by other means, make setting so that liquid delivery is stopped automatically if leakage occurs.

Precautions on Disposal of Waste Solution

- Be sure to collect waste solution and treat it properly for disposal. Improper disposal treatment of waste solution may result in environmental pollution.

Precautions on Accuracy/Precision of Measured Values

- Use control sample measurements to ensure that the performance of the instrument is normal.

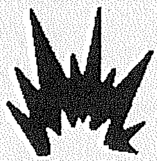
SAFETY SUMMARY

WARNING LABELS

The warning labels shown below are attached on the Model L-7485 fluorescence detector.

(1) Explosion of Vapor from Flammable Chemicals

WARNING



Beware of explosion!
Can cause serious injury.
High concentration of organic solvent vapor may lead to explosion.
Do not allow flammable chemicals to leak.

810-1952

[Attached position]
On right front side

(2) Electric Shock in Contact with Inside of Instrument

WARNING

Beware of electric shock!
Can cause death or serious injury.
Provide proper grounding connection.
Before removing instrument cover, unplug power cord.

810-1954

[Attached position]
On left rear side

SAFETY SUMMARY

(3) Electric Shock in Contact with Light Source Power Supply

WARNING

Beware of high voltage:
30 kV.
Can cause fatal or
serious injury.
Before removing light source
cover, turn power off and wait
for more than two hours.
Never release safety
mechanism inadvertently.

810-1959

[Attached position]
On right rear side

(4) Explosion of Xenon Lamp

WARNING

Beware of explosion!
Explosion of xenon lamp can
cause serious injury.
Refer to instruction manual
when handling xenon lamp.

810-1957

[Attached position]
On right rear side

SAFETY SUMMARY

(5) Burns in Touch with Hot Part

CAUTION

Beware of high temperature!
Can cause burns.
Light source lamp and lamp chamber remain hot for a while even after power-off.
Before lamp replacement, turn power off and wait until it becomes sufficiently cool.

810-1962

[Attached position]
On right rear side

FOREWORD

- This manual is intended for users of the L-7485 Fluorescence Detector and describes how to handle the instrument correctly.

- The L-7485 is a detector for liquid chromatography used widely for chemical analyses in fields such as pharmaceutics, chemistry, biotechnology, foodstuffs and the environment. Avoid using it for purposes other than liquid chromatography.

- For operation and maintenance of the instrument, read the instruction manual carefully and understand and observe the precautions.

- Keep this manual nearby so it can be referred to whenever needed.

- The contents of this manual are subject to alteration without prior notice.

MODEL L-7485
FLUORESCENCE DETECTOR

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Standard Operating Procedure SOP

1. INSTALLATION

1-1 Installation

1-1-1 Unpacking

⚠ **CAUTION**

Heavy Instrument!

This instrument weighs as much as 19 kg. Be careful not to drop it when carrying. Hold the front and rear parts of the instrument firmly when carrying.

NOTICE: Installation of this instrument at delivery shall be carried out by or under supervision of Hitachi's qualified service personnel or its approved service agent.
Prior to installation, please make preparatory arrangements for satisfying the installation requirements referring to this manual.
If relocation of this instrument becomes necessary after its initial installation, please notify your local Hitachi sales representative or nearest Hitachi service office.

Before unpacking, make sure that the bench top to be used is sturdy, level and can support the weight of the entire chromatographic system (approximately 50 kg, depending on the configuration).
Carefully unpack the instrument and place it on the bench top.

1-1-2 Checking Contents

After unpacking the system, carefully check the contents of each box against the packing list that is supplied.
If any part is missing or damaged, contact the nearest Hitachi dealer.

1-1-3 Laboratory Requirements

The instrument should be installed in a laboratory that meets the following requirements:

(1) Power Supply

Line voltage	100 to 115/220 to 240 V AC ± 10% of the rated voltage.
Frequency	50 or 60 Hz ± 4% of the rated frequency.
Power capacity	420 VA for the detector. The detector is used as part of an HPLC system, therefore the power to be provided to the system is dependent on the configuration of the system. Ensure that the power supply is sufficient to meet the needs of the entire system.

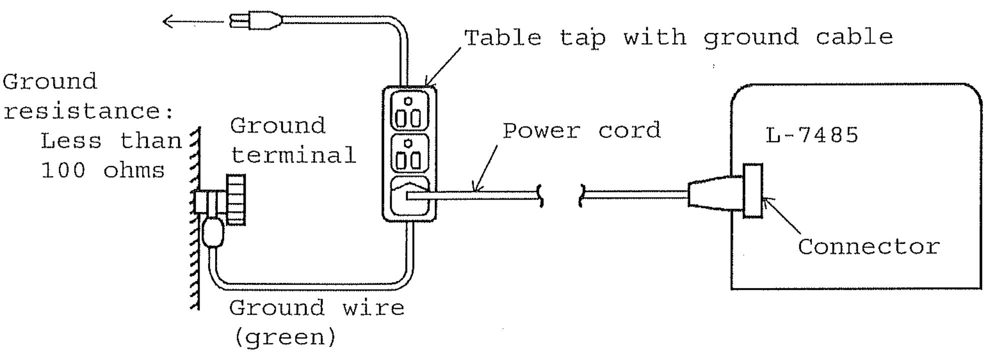
(2) Ground Terminal

⚠ WARNING

Ground Properly to Prevent Electric Shock Hazard!

- Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.
- This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.
- If a grounded 3-wire outlet is not available, then be sure to provide proper grounding connection.

It is required to provide grounding connection having resistance of less than 100 ohms within a distance of three meters.


Power outlet
Table tap with ground cable
Ground resistance: Less than 100 ohms
Ground terminal
Power cord
L-7485
Connector
Ground wire (green)

1-1-4 Installation Site

The detector should be installed in a location with the following dimensions:

Minimum width = 450 mm
Minimum depth = 650 mm

An open space of at least 150 mm should be provided to allow access to the detector.
The bench that is used for the instrument should be capable of supporting at least 50 kg.

1-1-5 Environmental Conditions for the Location of the System

The site for the system should meet the following conditions:

(1) Operating Temperature

The temperature of the laboratory should be between 4 and 35 °C. Ensure that the laboratory is free from significant variation in temperature (e.g. do not place the unit near an oven, air conditioner or a window).

(2) Operating Humidity

The humidity range should be between 45 to 85%.

(3) Air Quality Considerations

(a) The laboratory should have adequate ventilation.

(b) The atmosphere in the laboratory should not contain acidic or alkaline vapors or any gas that may corrode metal.

(c) The atomosphere in the laboratory should not contain vapors from organic solvents (in particular, benzene and paint thinner) which may affect the instrument painted surfaces.

(4) Additional Site Considerations

(a) Avoid placing the instrument near a window that will expose it to direct sunlight. If the unit is exposed to direct sunlight, performance may degrade due to temperature fluctuations. In addition, direct sunlight may discolor the painted surfaces of the instrument.

(b) Protect the unit against drafts. A draft may lead to significant fluctuations and may affect the performance of the instrument.

(c) Take care to ensure that the instrument is not subjected to strong vibrations or shocks.

(d) Avoid placing the instrument near equipment that radiates heat. Do not place the instrument near gas burners, electric heaters, or ovens.

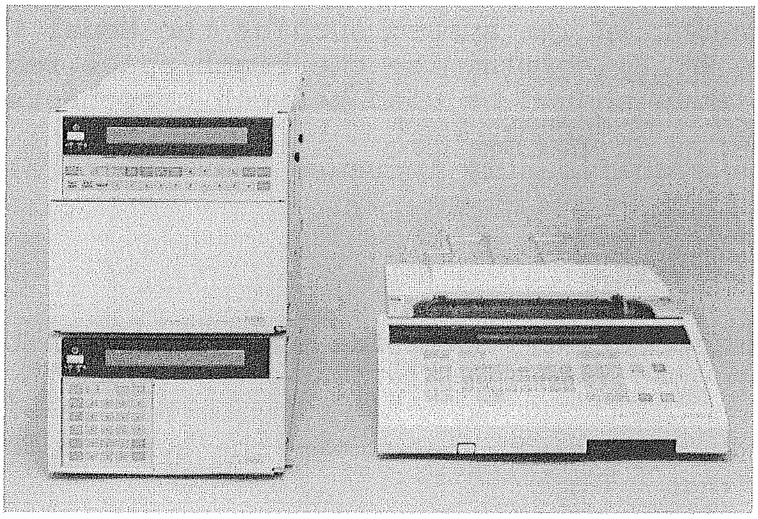
(e) Do not place your instrument near equipment that generates magnetic fields such as electric welding equipment, high frequency furnaces, line transformers, etc.

(f) Keep the area around the instrument clean. Dust may contaminate solvents or plug filters.

(g) The instrument should be connected to a source of electrical power that is free from sudden changes in the voltage or periodic fluctuations in the voltage. Sudden changes in the voltage may significantly increase detector noise.

(h) Avoid using units that include electric motors such as a stirrer or vibrator on the same power line as the instrument. If it is not possible to use a dedicated power line, ensure that a suitable noise reduction device is using.

1-2 System Configuration



C000016

Fig. 1-1 Example of System Configuration

1-3 Physically Connecting Components of the HPLC System

The Model L-7485 fluorescence detector can be located on top of other components in the system such as the Model L-7200 autosampler, etc., to minimize the bench space required. Fastening plates should be used between components as shown in Fig. 1-2.
These plates significantly increase the structural stability of the system.

To fasten the units:

(1) Remove appropriate cover fastening screw in the side panels of each unit (see Fig. 1-2).

(2) Place the fastening plate over the screw holes and re-insert the screws.

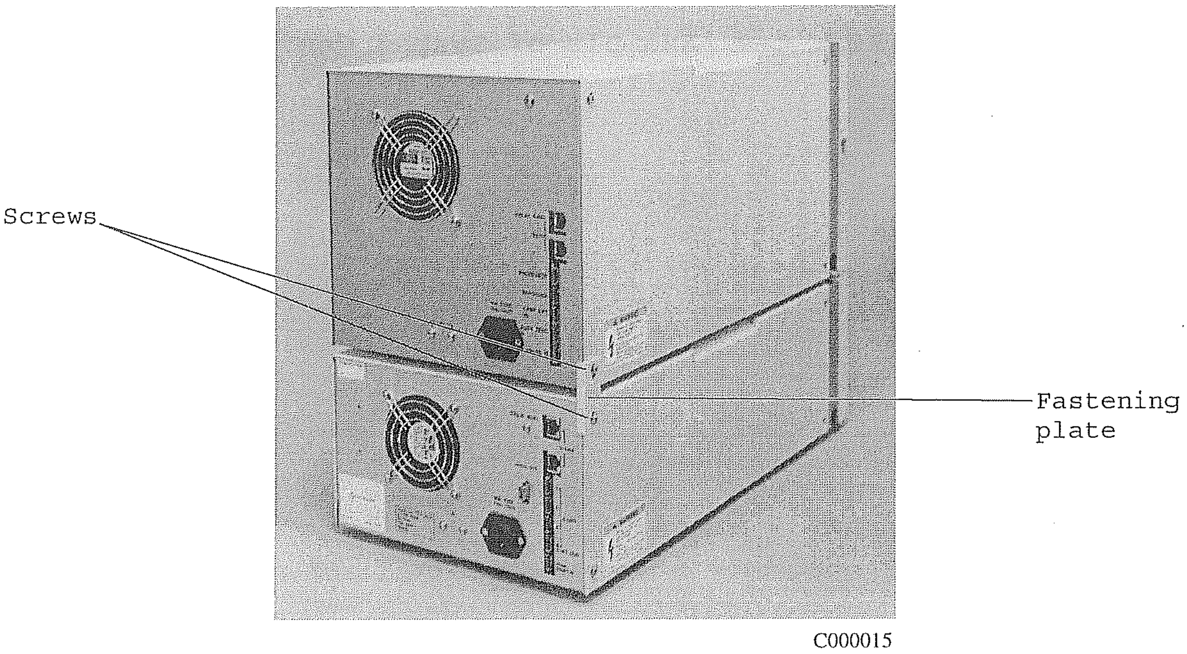


Fig. 1-2 Fastening between Component Units

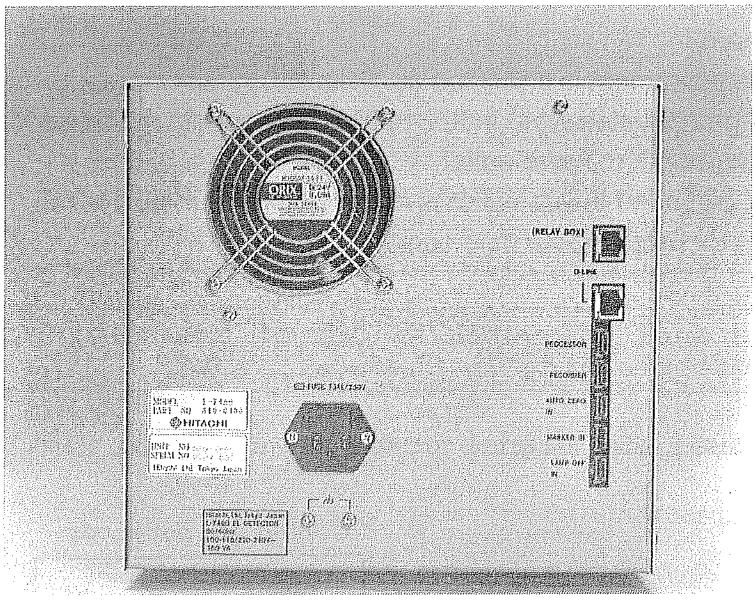
1-4 Setting up D-line Communication Function

The D-line is Hitachi’s unique communication network for automating a liquid chromatograph system. An automated liquid chromatograph system can be easily set up by connecting pumps and other accessories to a data processor or integrator via the D-line communication network.
For details about setting up the network, refer to “Communication Function Using D-line System” in Section 4-1.

1-5 Preparing the Instrument for Use

1-5-1 Check the Power Supply Voltage

Make sure the instrument voltage is set to the line voltage in your facility. The set instrument voltage is indicated on its rear panel.


(RELAY BOX)
PROCESSOR
RECORDER
AUTO ZERO IN
MARKER IN
LAMP OFF IN
HITACHI

C960545

⚠ WARNING

Using a Rapid Response Circuit Breaker

A rapid response circuit breaker will cut the current to the system in a few milliseconds if a significant increase in the current is detected (i.e. due to a short circuit). If such a circuit breaker is used, ensure that it is designed to operate at the line frequency to prevent personal injury or a fire hazard.

1-5-2 Wiring

⚠ WARNING

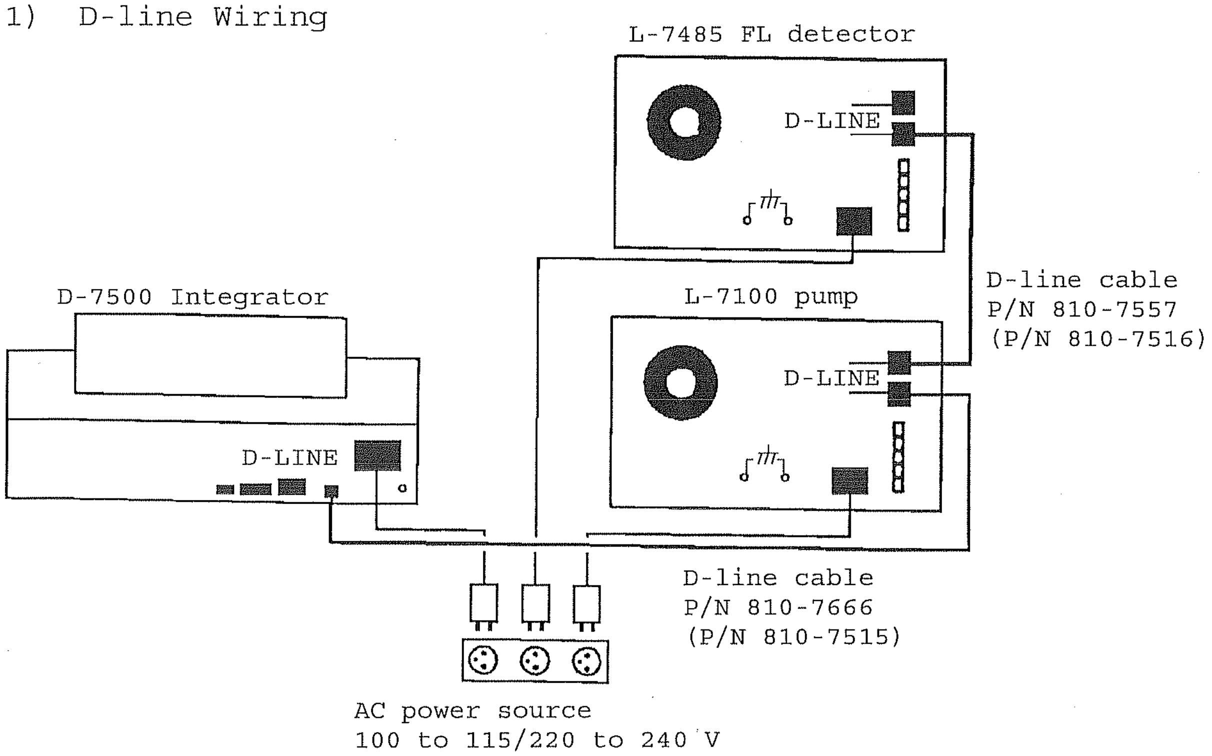
Ground Properly to Prevent Electric Shock Hazard!

- Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.
- This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.
- If a grounded 3-wire outlet is not available, then be sure to provide proper grounding connection.

NOTICE: Make sure the POWER switch of the detector is OFF before plugging in the power cord.

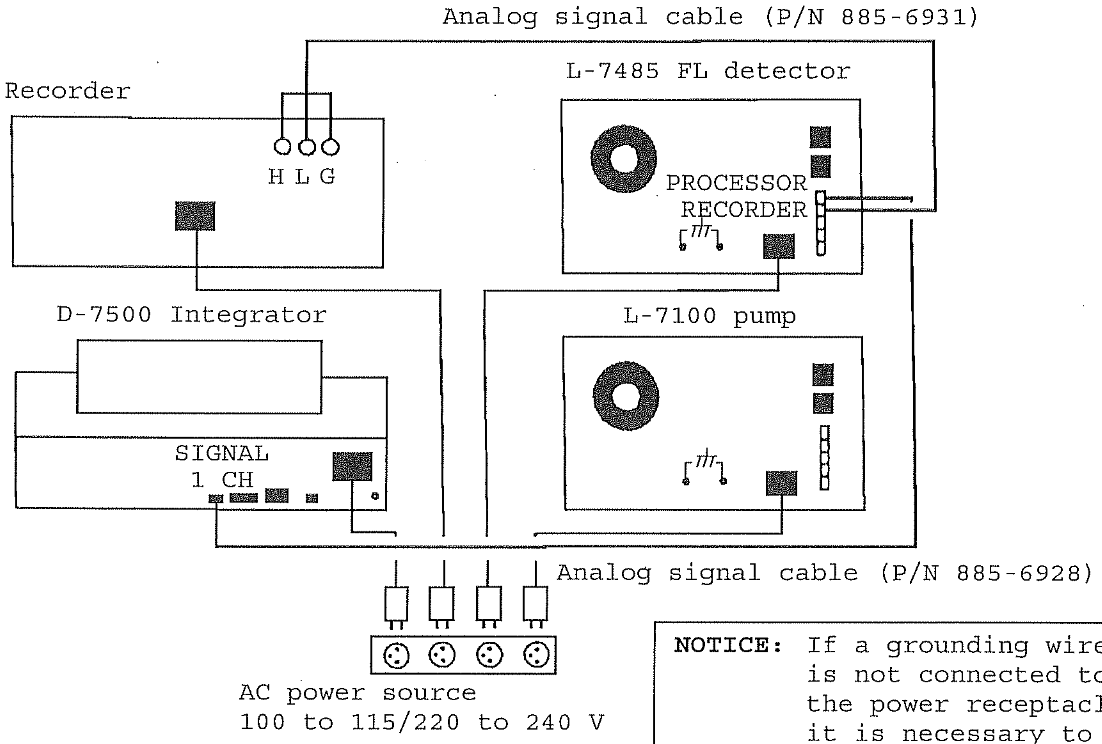
Connect the power and signal cords as shown in Fig. 1-3.

(1) D-line Wiring


L-7485 FL detector
D-LINE
D-7500 Integrator
D-LINE
L-7100 pump
D-LINE
D-line cable
P/N 810-7557
(P/N 810-7516)
D-line cable
P/N 810-7666
(P/N 810-7515)
AC power source
100 to 115/220 to 240 V

( ): For connection with the instrument bearing the CE conformity marking.

(2) Analog Input/Output Wiring


Analog signal cable (P/N 885-6931)
Recorder
H L G
L-7485 FL detector
PROCESSOR
RECORDER
D-7500 Integrator
SIGNAL
1 CH
L-7100 pump
Analog signal cable (P/N 885-6928)
AC power source
100 to 115/220 to 240 V

NOTICE: If a grounding wire is not connected to the power receptacle, it is necessary to connect the GROUND terminal at the rear panel of each unit to the grounding point.

Fig. 1-3 Wiring Diagram

1-5-3 Tubing

⚠ WARNING

Ignition of Flammable Chemicals!

- Beware of ignition hazard when using flammable chemicals such as organic solvents.
- Always check the following conditions. If an abnormality is found, stop operation immediately.
    - Leakage of solvent or waste solution
    - Leakage of solvent inside the instrument
    - Inadequate ventilation of the laboratory room
- This instrument is not explosion-proof. Although aqueous solvents or organic solvents having an ignition point of 70 °C or higher are usable, do not use organic solvents having an ignition point below 70 °C.
- When using flammable chemicals, be careful about possible ignition due to static electricity. Particularly when using non-conductive chemicals, employ a conductive vessel made of metal or the like and provide grounding connection correctly.

⚠ WARNING

Explosion of Vapor from Flammable Chemicals!

- If a flammable chemical such as organic solvent leaks from the flow path of the instrument and its vapor concentration exceeds the explosion limit, it may cause spontaneous combustion with dangerously explosive results.
- When using a flammable and readily volatile chemical, be sure to check for leakage from the instrument flow path and ventilate the laboratory room adequately.

* Inlet tubing-between the column outlet and the inlet of the detector.

* Drain tube-between the outlet of the detector and the waste bottle.

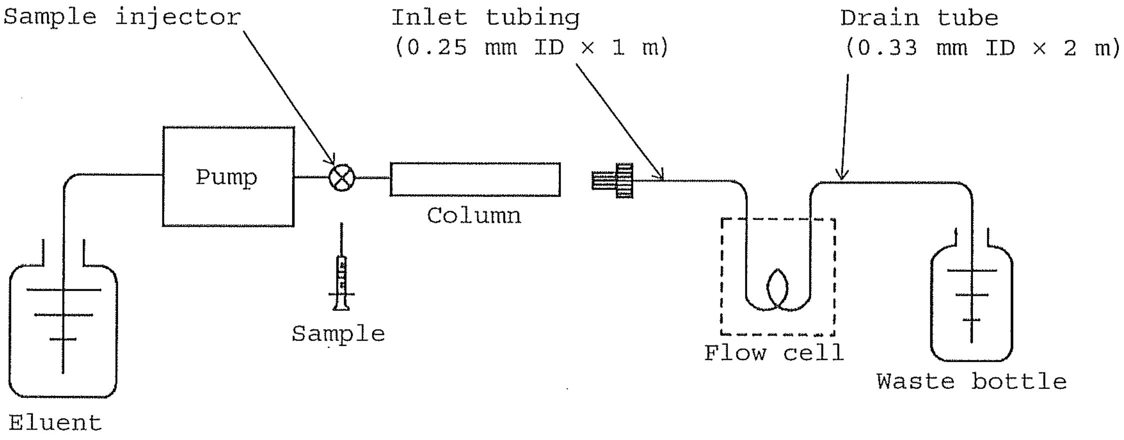

Sample injector
Inlet tubing
(0.25 mm ID × 1 m)
Drain tube
(0.33 mm ID × 2 m)
Pump
Column
Sample
Flow cell
Waste bottle
Eluent

Fig. 1-4 Tubing Diagram

The length of the tubing between the end of the column and the flow cell should be made as short as possible to minimize post column degradation of the chromatographic resolution.

1-5-4 Preparing the Unit for Operation

(1) Make sure the xenon lamp is mounted in place.

(2) Turn ON the power switch of the instrument. Verify that the LAMP indicator on the front panel is lit and the cooling fan is rotating.

NOTICE: Ozone (O_3) may be detected in the area around the detector immediately after the xenon lamp has been lit.
After a few minutes, the lamp house becomes warm enough to convert the ozone back to oxygen (O_2) and ozone will not be observed. This condition is normal and should not be a cause for concern. If the ozone persists for 5 minutes or longer, contact your Hitachi service representative.

2. FUNCTIONAL DESCRIPTION OF THE DETECTOR

2-1 Keyboard

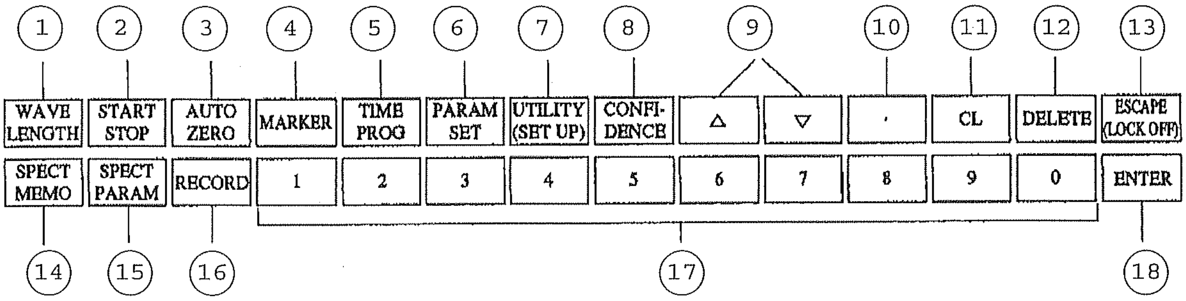

①
②
③
④
⑤
⑥
⑦
⑧
⑨
⑩
⑪
⑫
⑬
WAVE LENGTH
START STOP
AUTO ZERO
MARKER
TIME PROG
PARAM SET
UTILITY (SET UP)
CONFI-DENCE
△
▽
.
CL
DELETE
ESCAPE (LOCK OFF)
SPECT MEMO
SPECT PARAM
RECORD
1
2
3
4
5
6
7
8
9
0
ENTER
⑭
⑮
⑯
⑰
⑱

Fig. 2-1 Keyboard

All data entry is made via the keyboard on the front panel of the unit (Fig. 2-1). The role of each key is indicated below.
For additional information about a button, see the detailed discussions in Chapter 3.

2-1 Keyboard

No.	Indication	Function	Remarks
①	WAVE LENGTH	(Wavelength) Used to set the Excitation and Fluorescence Wavelengths.	See Section 3-3 (3)
②	START STOP	(Start, stop) (1) Starts(Stops) the time program. (2) Starts(Stops) analog output of the stored spectrum.	See Section 3-3 (7)
③	AUTO ZERO	(Auto zero) Sets the fluorescence intensity to zero at the indicated wavelength.	See Section 3-3 (4)
④	MARKER	(Marker) Places a mark on the recorder (when analog input/output option is used).	See Section 3-3 (5)
⑤	TIME PROG	(Time program) Selects and sets the time program	See Section 3-3 (6) 3-3 (13)
⑥	PARAM SET	(Parameter set) Used to select the time constants, pmt voltage, the desired time program, and lamp mode.	See Section 3-3 (2)
⑦	UTILITY (SET UP)	(Utility) (1) Sets offset value, output of measurement parameters via the D-line communication network, selects if lamp should be turned off at the end of analysis and accesses display mode. (2) If the utility key is depressed when the power switch is turned on, digital communication via the D-line is established. (SET UP)	See Section 3-3 (8) See Section 3-3 (1)
⑧	CONFI-DENCE	(Confidence) Used for setting the key lock, for checking lamp energy and wavelength accuracy and for accessing the Xe lamp logbook.	See Section 3-3 (12)

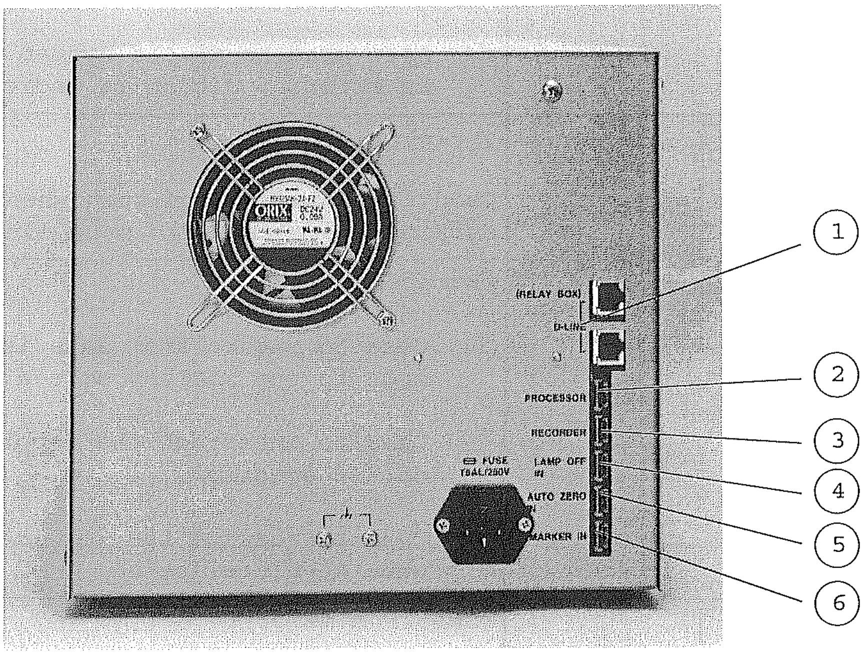
(cont'd)

No.	Indication	Function	Remarks
⑨	△ ▽	Accesses the next screen (or previous screen).	
⑩	•	Used for inputting a decimal point.	
⑪	CL	(Clear) (1) Used to remove the present data input on the display before the $\boxed{\text{ENTER}}$ key is pressed (e.g. to erase incorrect data). (2) Also used to re-set system when an error condition is indicated.	
⑫	DELETE	(Delete) Used for deleting one step when editing a program with many lines.	
⑬	ESCAPE (LOCK OFF)	(Escape) (1) Used to return from data input mode to the monitor screen. (2) Used to interrupt the recording of the spectrum. (3) Used to release the key lock when keys are locked.	
⑭	SPECT MEMO	(Spectrum memory) Obtains spectrum over a specified wavelength range and stores the spectrum in memory. See ⑮ to specify scanning conditions.	See Section 3-3 (10)
⑮	SPECT PARAM	(Spectrum parameter) Used to set up excitation scanning range and fluorescence scanning range.	See Section 3-3 (9)
⑯	RECORD	(Record) Used to specify the recorder full scale range, recorder output speed, and output data. Also used for setting the factors of recorder and spectral output.	See Section 3-3 (11)

(cont'd)

No.	Indication	Function	Remarks
⑰	0 ~ 9	Used for entering information	
⑱	ENTER	(Enter) Accepts the information that was entered.	

2-2 The Rear Panel


ORIX
(RELAY BOX)
D-LINE
PROCESSOR
RECORDER
FUSE
T6AL/250V
LAMP OFF
IN
AUTO ZERO
IN
MARKER IN
1
2
3
4
5
6

C000002

Fig. 2-2 Rear Panel

2-2

No.	Indication	Function	Remarks
①	D-LINE (*Note)	(1) Used to transmit the fluorescence signal (or the acquired spectral data) to other units on the D-line. Also, used to send/receive the instrument parameter data via the D-line.	(1) The fluorescence intensity data is output.
		(2) Used for input/output of each contact signal.	(2)
		(a) START IN	(a) The program is initiated.
		(b) STOP (ERROR) IN	(b) An error condition in another instrument is indicated to the L-7485 Detector.
		(c) STOP (ERROR) OUT	(c) An error condition on this instrument is indicated to other units on the D-line.
		(d) READY/BUSY OUT	(d) The operational status of the instrument is indicated to other units on the D-line.
②	PROCESSOR	Analog output for data processor (integrator) (1) The Fluorescence intensity is presented as a function of time (2) Stored spectral data	The fluorescence value data is presented as a function of wavelength.
③	RECORDER	Analog output to strip chart recorder (1) The fluorescence intensity is presented as a function of time (2) Stored spectral data	The fluorescence value data is presented as a function of wavelength.
④	AUTO ZERO IN	The auto-zero function is activated by means of contact signal input.	
⑤	MARKER IN	The marker function is activated by means of contact signal input.	The marker function is started when a short-circuit signal of more than one second is input.
⑥	LAMP OFF IN	The lamp is turned off by means of a contact signal input.	

(*Note) Functions (1) and (2) are selectable at setup.

2-3 Specifications

(1) Photometric principle : Ratio photometry; intensity of transmitted beam is monitored.

(2) Light source : 150 W xenon lamp

(3) Excitation wavelength setting range : 200 to 850 nm, and zero order

(4) Emission wavelength setting range : 250 to 900 nm, and zero order
Note that photomultiplier must be changed at emission wavelengths greater than 731 nm.

(5) Excitation side spectral bandwidth : 15 nm

(6) Emission side spectral bandwidth : 15,30 nm(variable)

(7) Wavelength setting communication : By keyboard or through

(8) Wavelength accuracy : ±3 nm

(9) Wavelength repeatability : ±0.5 nm

(10) Recorder output : 10 mV full scale
Full scale range of 1 to 1000 is settable in increments of 1.

(11) Processor output : 1 V full scale; digital output of -40 (-40 mV) to 1000 (1000 mV) via D-line.

(12) Response : Changeable in 5 steps, corresponding to time constants of 0.1, 0.5, 2.0, 4.0, 8.0 seconds.

(13) Sensitivity : S/N ratio of 350 or higher with Raman spectrum of water (wavelength: 350 nm, time constant: 2.0 s)

(14) Auto zero range : 0 to 1000

(15) Offset : 0 to 1000 (settable in steps of 1)

(16) Spectrum memory : Up to 8 excitation or emission spectra can be stored.

(17) Marker : Marker output of approx. 0.6 mV at recorder terminal (corresponding to about 6 graduations on chart)

(18) Instrument parameter setting : Following parameters are settable and stored in battery-backed-up memory when power is turned off.

- Full scale range
- Spectrum measuring range
- Photomultiplier applied voltage
- Offset value

(19) Time program :

No. of programs ; 9
Settable time ; Up to 600 minutes in increments of 0.1 minute
No. of steps storable ; Up to 100 steps for a total of 9 files
Programmable items ; Excitation wavelength, emission wavelength, baseline processing

(20) Display function : 40 characters × 2 lines on backlighted LCD

(21) Communication function : D-line communication

(22) External output terminals : Analog output

- For processor ; 1 V full scale
- For recorder ; 10 mV full scale

(23) External I/O contact terminals : Time program start, error input/output, busy output (Above contacts are incorporated in D-line connector, and effective with analog connection.)
Auto zero input, marker input, lamp off input

(24) Flow cell capacity : Standard cell 12 µL (irradiated capacity)

(25) Flow cell withstand pressure : 2.9 MPa

(26) Operating temperature range : 4 to 35 °C

(27) Operating humidity range : 45 to 80% RH (non-condensing)

(28) Power requirement : 100-115, 220-240 V AC, 50/60 Hz

(29) Power consumption : 420 VA

(30) Dimensions : 260 mm W × 500 mm D × 230 mm H

(31) Weight : Approx. 19 kg

PRECAUTIONS ON CE CONFORMITY MARKING

1. ELECTROMAGNETIC INTERFERENCE

This equipment conforms to the EC Directives 89/336/EEC and has the CE conformity marking. It is designed to satisfy the European Norms EN55011 (Mar. 1991) and EN50082-1 (Jan.1992).

This equipment is classified as Group 1-Class A of EN55011. Class A equipment is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purpose.
This equipment must not be used in domestic establishments nor in establishments directly connected to a low voltage power supply network which supplies buildings used for domestic purpose.

This equipment is also designed to comply with the European Norm EN50082-1. If the equipment is used near an intense electromagnetic source, however, interfering noise may be given to the instrument to cause an adverse effect on its performance or functionality.

2. SAFETY

This equipment also conforms to the EC Directive 73/23/EEC and has the CE conformity marking. It is designed to satisfy the European Norms EN61010-1 (Mar. 1993).

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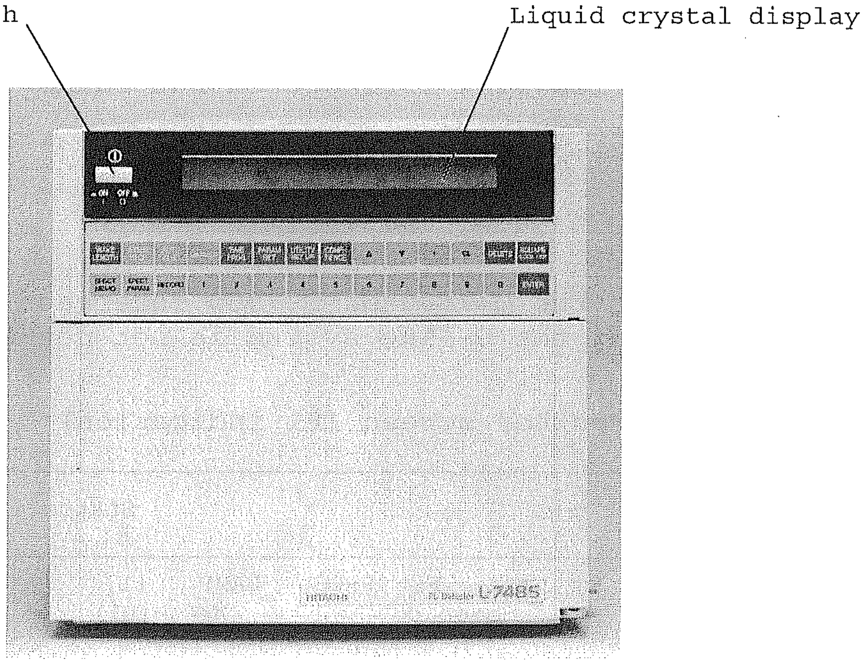
3. OPERATING THE DETECTOR

3-1 Turning on the Power

Turn on the POWER switch of the detector.



C000001

An initialization test is carried out when the unit is powered up. During the initialization period, the lamp is ignited, the monochromators are set and the system is autozeroed.

NOTICE: The sequence of events described in this section are automatically performed when the instrument is powered up. If the monitor display screen does not appear on the LCD within 3 minutes after turning on the power, or if an error message appears on the LCD, refer to "Troubleshooting" in section 6-7 or "Error Messages" in section 6-8. But, if the unit is powered up again with the excitation or emission wavelength set to 0-order light, it will take about 5 minutes for the monitor display screen to appear.

- Liquid Crystal Display (LCD)

```
FL Detector   8107064-00
Copyright (C) Hitachi, Ltd.  2000
```

After a short period of time, the monitor display presents:

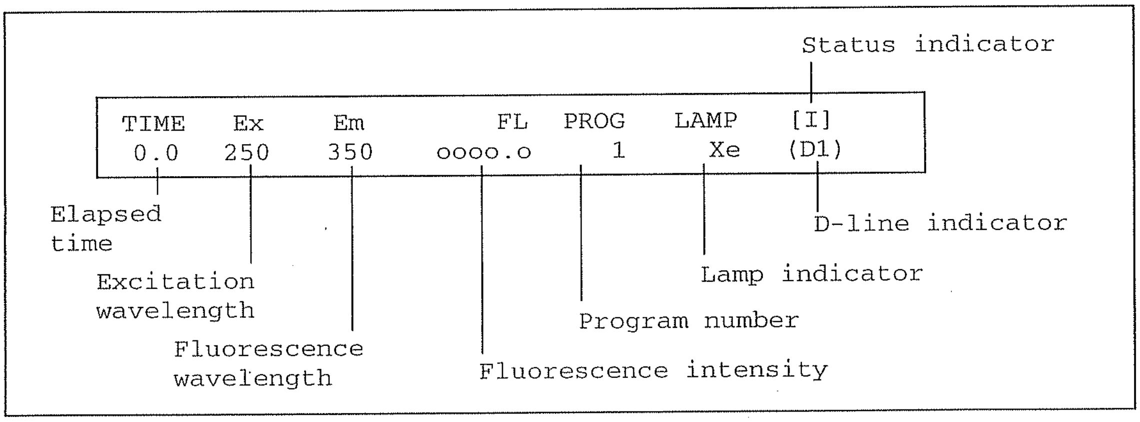
```
WL DRIVE & LAMP INITIALIZING
                                        (D1)
```

The sequence of initializing the monochromator, turning on the lamp and auto zeroing the instrument is performed during this period. If these steps are successfully completed and the D-line communication link is expected but cannot be made, the message will be presented.

```
D-LINE SYSTEM IS NOT READY
                                        (D1)
```

This screen appears to indicate that this detector is waiting for connection to the D-7500 or D-7000 interface module and will not appear if the D-line system is not connected to the system.

Monitor screen (FL indication)


Status indicator
TIME Ex Em FL PROG LAMP [I]
0.0 250 350 oooo.o 1 Xe (D1)
Elapsed time
D-line indicator
Excitation wavelength
Lamp indicator
Program number
Fluorescence wavelength
Fluorescence intensity

(1) The currently selected program number is indicated.

(2) 'Xe' is indicated when the xenon lamp is turned on, and 'OFF' is indicated when the lamp is turned off.

(3) The D-line indicator describes the present condition of the D-line communication mode; 'D1' is indicated when channel 1 selected, 'D2' is indicated when channel 2 is selected, and 'A' is indicated when analog connection is selected.

(4) The status indicator describes the present status of the system. 'I' is indicated when the detector is in the initial status, 'R' is indicated when it is in the time program running status, 'B' is indicated when it is in the busy status, and 'L' is indicated when it is in the key-locked status.

After the initialization process is complete, the unit should be allowed to warm up for approximately 30 minutes.

3-2 General Information about Entering Parameters

(1) Use the Numeric keys and the ENTER key to enter a value.

(2) The CL key is used to erase the present data entry (e.g., if you have made an error in entering data, but have not pressed the ENTER key).

(3) If the present settings are acceptable, simply press the ENTER key or the ∇ key to access the next display. The Δ or ∇ arrow keys are used to access the previous or next display.

(4) After you have finished editing a display, use the ESCAPE key to return to the monitor screen.

(5) If the time program is not in use, spectral output can be performed at any time. If spectral output is started during data acquisition, the data is automatically directed to the integrator.

3-3 Operation

(1) Selecting the Digital Communication Channel via the D-line Communication Link

To access the Digital Communication set-up screen when powering up the instrument, press:

[POWER] + [UTILITY (SET UP)] (⑦ SET UP)

at the same time.

Input

(A) Call up the D-line digital communication setup screen by pressing the utility key when powering up the instrument.

[POWER] + [UTILITY (SET UP)] key

(B) The initial screen will be presented:

```
FL Detector     8107064-00
Copyright (c)   Hitachi,Ltd. 2000
```

(C) After the initial screen is presented, the display will present the various D-line digital communication options.

```
D-LINE:  OFF=0,   ON(CH1)=1,   ON(CH2)=2
       0
```

Select the desired value and press → [ENTER] .

(D) Turn off the power to the system and then turn the power back on. The D-line communication is now active.

Explanation

(A) Press the POWER switch while holding down the UTILITY (SET UP) key (at power-on). The D-line digital communication setup screen will then appear.

(B) The currently set value is indicated. (The default value is 0.)

Input Value	**Meaning**
0	The D-line communication link is not established
1	D-line communication is established via channel 1
2	D-line communication is established via channel 2

When the system is turned on again, the specified communication link is automatically made as part of the initialization.
After the value is entered, the monitor screen is presented.

(2) Setting up the Instrument Conditions

PARAM SET (⑥ Parameter set)

Input

(A) Call up the instrument condition setup screen by pressing the PARAM SET key.

PARAM SET key

(B) Specify a time constant.

```
TIME CONSTANT    (0.1s=1,  0.5s=2,  2.0s=3,
3                 4.0s=4,  8.0s=5)
```

Select the desired value and press → ENTER →
The photomultiplier voltage setup screen will be presented. (If the indicated time constant is acceptable, simply press the ∇ key to go to the photomultiplier voltage setup screen, or press the Δ key to go to the monitor screen.
As an alternative, press the ESCAPE key to return to the monitor screen without changing the current setting.)

(C) Specify a Photomultiplier (PMT) voltage.

```
PMT VOLTAGE  (HIGH=1,  MED=2,  LOW=3)
1
```

Select the desired value and press → ENTER → The Em bandwidth setting screen will be presented.
(If the indicated PMT voltage is acceptable, simply press the ∇ key to go to the Em bandwidth setting screen, or press the Δ key to go to the time constant screen.
Press the ESCAPE key to return to the monitor screen without changing the current setting.)

(D) Specify an emission bandwidth.

```
Em BANDWIDTH  (STANDARD=1, WIDE=2)
1
```

Select the desired value and press → ENTER → The time program screen will appear.
(If the indicated setting is acceptable, simply press the ∇ key to go to the time program screen, or press the Δ key to go to the photomultiplier voltage setup screen.
Press the ESCAPE key to return to the monitor screen without changing the current setting.)

Explanation

(A) To call up the instrument condition setup screen, press the PARAM SET key.

The instrument parameters include:

- TIME CONSTANT
- PMT VOLTAGE (photomultiplier voltage)
- Em BANDWIDTH (emission bandwidth)
- USE TIME PROGRAM (selection of use/non-use of time program)
- LAMP MODE (selection of lamp turn-on/off)

(B) The current time constant value is displayed. (The default value is 3.) A time constant value ranging from 1 to 5 can be entered using the Numeric keys.

(C) The current PMT output range value is displayed. (The default value is 2.) An output range value of 1 to 3 can be entered using the Numeric keys.

(D) The current bandwidth setting of the emission monochromator is displayed. (The default value is 1.)

Input Value	**Meaning**
1	Emission monochromator bandwidth is standard (15 nm).
2	Emission monochromator bandwidth is wide (30 nm).

Input

(E) Indicate if the time program should be used.

```
USE TIME PROGRAM (NO=0,   YES=1)
1
```

Select the desired value and press → ENTER →
The lamp select screen will be presented.
(If the indicated time constant is acceptable, simply press the ∇ key or the ENTER key to present the lamp select screen, or press the Δ key to go to the Em bandwidth setting screen.
Press the ESCAPE key to return to the monitor screen without changing the current setting.)

(F) Selecting the Lamp Mode.

```
LAMP MODE  (OFF=0,   ON=1)
1
```

Select the desired value and press → ENTER →
The monitor screen will be presented.
(If the indicated time constant is acceptable, simply press the ∇ key or the ENTER key to present the monitor screen, or press the Δ key to go to the time program screen.)

Explanation

(F) The currently specified lamp mode value is displayed on the monitor screen. (The default value is 1.)

Input Value	Meaning
0	The lamp is turned off.
1	The lamp is turned on.

(3) To call up the Wavelength Range Setup screen, press wavelength.

WAVE LENGTH (① Wavelength)

Input

(A) Call up the wavelength setup screen.

WAVE LENGTH key

(B) Specify an excitation wavelength.

```
 Ex     Em     (200-850,  0) nm
250    350
```

Numeric key → ENTER → Fluorescence wavelength setup screen
(If the indicated excitation wavelength is acceptable, simply press the ∇ key to go to the fluorescence wavelength screen, or press the Δ key to return to the monitor screen. As an alternative, press the ESCAPE key to return to the monitor screen without changing the preset setting.)

(C) Specify a fluorescence wavelength.

```
 Ex     Em     (250-900,  0) nm
250    350
```

Numeric key → ENTER → Monitor setup screen

(If the indicated fluorescence wavelength is acceptable, simply press the ∇ key to go to the monitor screen, or press the Δ key to return to the excitation wavelength screen. As an alternative, press the ESCAPE key to return to the monitor screen without changing the current setting.)

(4) To adjust the Auto Zero

AUTO ZERO (③ Auto zero)

Input

(A) Press the AUTO ZERO key to adjust the FL intensity to zero.
This will affect the reading on the monitor screen and the output signal level.

```
TIME    EX    EM        FL    PROG   LAMP   [I]
 0.0   250   350   oooo.o       1     Xe   (D1)
```

Explanation

(A) To call up the wavelength setup screen, press the WAVE LENGTH key.

(B) The currently specified excitation wavelength is displayed. (The default value is 250 nm.) The desired excitation wavelength (input value) can be entered (range = 200 to 850 nm).

(C) The currently specified fluorescence wavelength is displayed. (The default value is 350 nm.) The desired fluorescence wavelength (input value) can be entered (range = 250 to 900 nm).

Explanation

(A) To correct for baseline drift or if an adjustment to the zero setting is desired, press the AUTO ZERO key to reset the output value to zero.

Auto zero adjustment range: 0 to 1000

If the auto zero range is exceeded, an error message is issued. (Refer to Section 6-8.)

(5) To place a Marker on the recorder

MARKER (④ Marker)

Input

(A) Press the MARKER key to indicate a marker line on the recorder.

(6) To select the Time Program

TIME PROG (⑤ Time program)

Input

(A) Call up the time program select/setup screen.

TIME PROG key

(B) Select a time program.
A time program is used when the excitation and/or emission wavelength should be changed during the run.
After the wavelength is changed, the baseline can be autozeroed or held as desired.
Up to 9 programs can be stored and up to 100 steps can be included in the time programs (e.g. one program can use 100 steps, or 9 programs can have 11 steps, etc.).

```
TIME PROGRAM NO.   (1-9)
1
```

Numeric key → ENTER key

Enter the code number for the program to be created or modified.
The relevant execution program number is changed at the same time.
(To return to the monitor screen without changing the value, press the ESCAPE key.)

(C) When a program is selected, it is necessary to specify whether it should be modified or a new program should be created.

```
MODIFY=0,  NEW=1
0
```

Numeric key → ENTER key

(To return to the monitor screen without changing the setting, press the ESCAPE key.)

Explanation

(A) When the MARKER key is pressed, a marker of approximately 0.6 mV is output on the recorder terminal signal. This marker can be used to indicate the time when the sample is injected or when a change in a present condition is made during measurement.

Explanation

(A) To call up the time program select/setup screen, press the TIME PROG key.

The time program is used to automatically change the wavelength during analysis, specify the time for the change of excitation wavelength, fluorescence wavelength and baseline.

(B) The currently selected program number is displayed. (The default value is 1.) The acceptable range is from 1 to 9. Up to nine time program files can be set up, and up to 100 steps (see Section 3-3-6 (D) for information on entering lines) can be specified in a total of nine files. The time programs are held in memory by battery backup when power to the system is turned off.

(C) The default value is 0. This value will be indicated whenever a program is selected. A value of 0 or 1 can be entered using the keypad.

Input Value	**Meaning**
0	MODIFY Enter '0' to modify or add lines to a stored time program.
1	NEW Enter '1' to delete the present stored time program and create a new one.

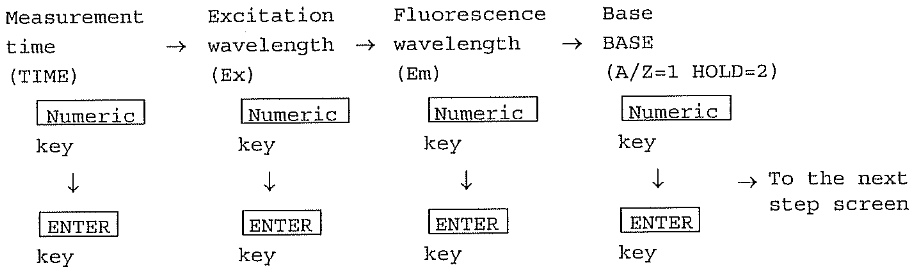
Input

(D) Set up the time program.
The first line of the time program is indicated below.

```
TIME    Ex   Em    BASE (A/Z=1, HOLD=2)
_ 0.0   250  350      1
```

Cursor movement

Enter the appropriate time (the time for the first line is fixed at 0.0) and press ENTER to place the cursor in the Excitation Wavelength field. Enter the desired excitation wavelength and press ENTER to place the cursor in the Fluorescence (Em) field.
After the fluorescence wavelength is selected, the cursor will appear in the BASE field. Enter 1 to indicate if the autozero feature should be used to re-set the baseline or enter 0 to indicate if the present level of the baseline should be maintained when the wavelength is changed.


Measurement time (TIME)
Excitation wavelength (Ex)
Fluorescence wavelength (Em)
Base BASE (A/Z=1 HOLD=2)
Numeric key
Numeric key
Numeric key
Numeric key
ENTER key
ENTER key
ENTER key
ENTER key
To the next step screen

(a) Input of measurement time
(b) Input of excitation wavelength
(c) Input of fluorescence wavelength
(d) Input of baseline processing

NOTICE 1: In the time program, the 'TIME' for the first step is 0.0, which cannot be changed or deleted. After this step has been edited, the time field for the second step can be accessed by using the ▽ key. At this point, any time up to 600.0 minutes can be used. Continue to enter lines. The time program can be carried out until the end-of-measurement time specified at the last step is reached.

NOTICE 2: The time interval between lines should be at least 0.3 min.

Explanation

(D) The currently specified values for each setting are displayed.

- (a) Measurement time (TIME)
- (b) Excitation wavelength (Ex)
- (c) Fluorescence wavelength (Em)
- (d) Baseline processing (BASE)

(a) Measurement time input range (The default value is 0.0.)
The range of the measurement time is from 0 to 600 minutes in increments of 0.1 minute.

(b) Excitation wavelength input range (The default value is 250 nm.)
The range of the excitation wavelength is from 200 to 850 nm and 0 (zero order).

(c) Fluorescence wavelength input range (The default value is 350 nm.)
The range of the fluorescence wavelength is from 250 to 900 nm and 0 (zero order).

(d) Baseline processing input range (The default value is 1.)

Input Value	**Operation**	**Baseline Processing**
1	Auto Zero	Auto zero setting should be performed after the wavelength is changed.
2	Hold	The baseline is held after the wavelength is changed. The 'Hold' function can be turned off during a run by pressing the auto zero setting.

- To erase a numeric value that was selected but not entered, use the CL key (⑪ Clear).
- To remove a stored numeric value, use the DELETE key (⑫ Delete).
- To go to the previous or next step, use the Δ or ∇ key.
- After the final step is set up, press the ESCAPE key (⑬ Escape) to return to the monitor screen.

(7) Executing the Time Program

START STOP (② Start, stop)

Input

(A) Start the time program that has been set up by the TIME PROG described in 3-3 (6).

START STOP key

(B) To stop execution of the time program, press the START STOP key.

START STOP key

(8) Setting up the Utilities

UTILITY (SET UP) (⑦ Utility)

Input

(A) Access the UTILITY setup screen.
The Utility screen provides access to the selection of a number of features including setting the signal offset value, indicating whether the parameters should be printed with the chromatogram, if the lamp should be automatically shut off when the D-line system is turned off and selection of the active (displayed) parameter (e.g. the fluorescence intensity or the position of one of the monochromators).

UTILITY key

(B) Select the desired utility item for editing.

```
UTILITY:      OFFSET=1,     PRINT=2,
<1-4> _1      LAMP UTIL=3, DISPLAY MODE=4
```

Numeric key → ENTER key

Explanation

(A) To start the time program, press the START STOP key.

The status indicator 'R' appears.

(B) To stop the time program, press the START STOP key.

Explanation

(A) To access the utility setup screen, press the UTILITY key.

Settable items:

- (a) Offset value
- (b) Printout condition
- (c) Lamp turn-off enable/disable condition
- (d) Setting of monitor display mode

(B) The currently specified values are displayed. (The default value is 1.)

Input Value	**Meaning**
1	OFFSET Enter '1' for setting of offset value.
2	PRINT Enter '2' for selection of printout condition. This function is effective only when the D-line communication is turned on.
3	LAMP UTIL (Lamp utility) Enter '3' for setting of lamp turn-off enable/disable condition.
4	DISPLAY MODE (Signal mode) Enter '4' for setting of monitor display mode.

3-3 Operation

Input

(a) Specify an offset value.

```
OFFSET (0-1000)
_    0
```

Numeric key → ENTER key

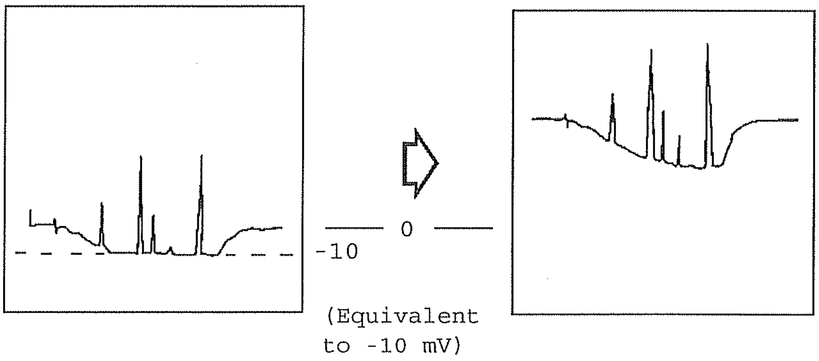
Explanation

(a) The currently specified value is displayed. (The default value is 0.)
The offset value adds a specified input signal value to the actual measured value on the output.

Offset setting range: 0 to 1000

The offset value can be specified in increments of 1.

Typically, the negative input limit or an integrator or data processor is approximately -10 mV. If the baseline decreases below this level, it becomes impossible to integrate the peaks in the chromatogram (see Fig. 3-1 (a)). In such a case, the offset function is used to adjust the shifted baseline to the allowable input signal range of the data processor (see Fig. 3-1 (b)).



(a) Offset Not Used (b) Offset Used

Fig. 3-1 Example of Offsetting

Input

(b) Specify the data to be printed out.

```
PRINT OUT  (PARAMETER=1,  LOGBOOK=2)
0
```

Numeric key → ENTER key

(c) Set the lamp turn-off enable/disable condition.

```
TURN OFF LAMP BY END SIGNAL
0       (NO=0,  YES=1)
```

Numeric key → ENTER key

(d) Set the monitor display mode.

```
DISPLAY MODE  (FL=1,  Ex<M>=2,  Em<M>=3)
1
```

Numeric key → ENTER key

Explanation

(b) The currently specified value is displayed.
(The default value is 0.)
Specify the type of data to be presented on the integrator.

Input Value	**Meaning**
0	Default value (0) Enter '0' to return to the monitor screen.
1	PARAMETER Enter '1' to output parameter data.
2	LOGBOOK Enter '2' to output logbook data.

NOTICE: This function is active only when the D-line communication is turned on.

(c) The currently specified value is displayed.
(The default value is 0.)
The lamp is turned off when the D-line all end signal or the error contact signal is received.

Input Value	**Meaning**
0	The lamp is not to be turned off.
1	The lamp is turned off when the D-line end signal or the contact error signal is received.

(d) The currently specified value is displayed.
(The default value is 1.)
Specify the monitor display mode.

Input Value	**Meaning**
1	FL mode Enter '1' for fluorescence intensity measurement. (Normal monitor screen)
2	Ex<M> mode Enter '2' for excitation energy check.
3	Em<M> mode Enter '3' for fluorescence energy check.

This option is used mainly for the wavelength accuracy check of monochromator(see 6-4).

(9) Setting up Spectrum Acquisition Parameters and Output

The spectrum Acquisition Parameters and Output window is used when an excitation or fluorescence spectrum is to be collected.

SPECT PARAM (⑮ Spectrum parameters)

Input

(A) Set parameters for acquisition of the spectrum.

SPECT PARAM key

(B) Select an acquisition (scan) mode.

```
SCAN MODE  (Ex=1,  Em=2)
2
```

Numeric key → ENTER key

NOTICE: The system will present either the Ex SCANNING WL RANGE screen (C) or the Em SCANNING WL RANGE (D) when ENTER is selected.

(C) Specify an excitation scan range.

(a) Indicate the starting wavelength (minimum value).

```
Ex SCANNING WL RANGE  (200-800) nm
200 - 550                       1 nm step
```

Enter the desired wavelength and press the ENTER key to change the value.
Use the Δ or ∇ key if the present value is acceptable. The Δ returns to the monitor screen, while the ∇ key accesses the Ex SCANNING WL RANGE end wavelength field.

(b) Indicate the ending wavelength (maximum value).

```
Ex SCANNING WL RANGE  (250-550) nm
200 - 550                       1 nm step
```

Enter the desired wavelength and press the ENTER key to change the value.
Use the Δ or ∇ key if the present value is acceptable.
The Δ returns to the Ex SCANNING WL RANGE start field, while the ∇ key access the monitor screen.

Explanation

(A) To set the excitation and fluorescence scan ranges (maximum and minimum value), press the SPECT PARAM key.

The maximum wavelength scan range (= end wavelength - start wavelength) is 350 nm.

(B) The currently specified value is displayed. (The default value is 2.)

Input Value	Scan Mode	Meaning
1	Ex	Enter '1' to define the excitation scan range.
2	Em	Enter '2' to define the fluorescence scan range.

(C) The information in this section is not relevant in Em Scan Mode.

(a) The currently specified value is displayed.
(The default value is 200.)
The allowable input range is from 200 to 800 nm.

(b) The currently specified value is displayed.
(The default value is 550.)
The allowable input range is "start wavelength + 50" to "start wavelength + 350".

Input

(D) Specify a fluorescence scan range.

(a) Indicate the starting wavelength (minimum value).

```
Em SCANNING WL RANGE  (250-850) nm
250 - 600                      1 nm step
```

Enter the desired wavelength and press the ENTER key to change the value.
Use the Δ or ∇ key if the present value is acceptable.
The Δ returns to the monitor screen, while the ∇ key accesses the Em SCANNING WL RANGE end wavelength field.

(b) Indicate the ending wavelength (maximum value).

```
Em SCANNING WL RANGE (300-600) nm
250 - 600                      1 nm step
```

Enter the desired wavelength and press the ENTER key to change the value.
Use the Δ or ∇ key if the present value is acceptable.
The Δ returns to the Em SCANNING WL RANGE start field, while the ∇ key accesses the monitor screen.

(10) Collecting the Spectra

SPECT MEMO (⑭ Spectrum memory)

Input

(A) To collect the spectra.
SPECT MEMO key

(B) Specify a storage file.

```
SPECTRUM MEMORY NO.  (1-7)    MODE:Em
No.  _
```

Numeric key → ENTER key

(C) While the unit is scanning, the monitor screen will present:

```
SCANNING SPECTRUM NO.  o
Ex:ooo     Em:ooo
```

To cancel spectral data acquisition, press the ESCAPE key.

NOTICE: The spectrum will not be saved if the ESCAPE key is pressed.

Explanation

(D) The information in this section is not relevant in Ex Scan Mode.

(a) The currently specified value is displayed. (The default value is 250.) The allowable input range is from 250 to 850 nm.

(b) The currently specified value is displayed. (The default value is 600.) The allowable input range is "start wavelength + 50" to "start wavelength + 350".

NOTICE: The maximum wavelength scan range (= end wavelength - start wavelength) for both excitation and emission is 350 nm.
For spectral acquisition in a range beyond 350 nm, it should be divided into two times for measurement.

Explanation

(A) When the SPECT MEMO key is pressed, the appropriate monochromator is scanned over the range which was set in Section 3-3 (9) and the spectrum is stored using the indicated memory number.
When collecting a spectrum, there should be a sample in the cell and the flow rate should be zero (0).

(B) The currently specified value is displayed. (None is indicated by default.)
Up to seven spectra can be stored. The currently selected scan mode is displayed by the MODE field.
Spectral memory numbers (input values) are used for identification of stored spectra. When a spectral memory number is specified (input), the spectrum in the memory location is deleted and the new spectrum data is stored.

(C) The specified spectrum number and the current wavelength are displayed. After the spectrum has been stored, the monitor screen is presented. The stored spectral data is not retained when power is turned OFF.

NOTICE: Stored spectral data is not retained when the power is turned OFF.

(11) Setting the Recorder and Spectral Output Factors

RECORD

(⑯ Record)

Input

(A) Access the recorder/spectral output factor setup screen by pressing RECORD .

RECORD key

(B) Record is used to access several screens which are used to indicate information about the recorder and output of the spectrum.

```
RECORD:  RCD RANGE=1, RCD SPEED=2,
<1-3>  1 SPECT RCD=3
```

Numeric key → ENTER key

(a) Indicate the full-scale range of the recorder for analog output.

```
RECORDER RANGE (1-1000)
1000
```

Numeric key → ENTER key

(b) Indicate the output speed for the recorder.

```
RCD SPEED  (40 nm/min=1, 60 nm/min=2)
2
```

Numeric key → ENTER key

(c) (i) Assign a spectral number to output data.

```
RECORD SPECTRUM NO. (1-8)
1
```

Numeric key → ENTER key

(ii) Specify background data.

```
BACKGROUND SPECTRUM NO. (0-8)
0
```

Numeric key → ENTER key

Explanation

(A) When the RECORD key is pressed, the recorder/spectral output factor setup screen will then appear. This screen is used to set:

(a) The recorder full scale range
(b) The recorder output speed
(c) The output data

(B) The currently specified recorder full scale range is displayed. (The default value is 1.)

Input Value	**Meaning**
1	RCD RANGE (Recorder range) Enter '1' to set recorder output full scale range.
2	RCD SPEED (Recorder speed) Enter '2' to set recorder output speed.
3	SPECT RCD (Spectral recorder) Enter '3' to set output data format.

This is used to indicate if you want to present the observed spectrum, or want to subtract a background spectrum.

(a) The currently specified recorder range is displayed. (The default value is 1000.)
A value ranging from 1 to 1000 can be entered using the Numeric keys.

(b) The currently specified recorder speed is displayed. (The default Recorder Speed value is 2.)

Input Value	**Meaning**
1	40 nm/min=1
2	60 nm/min=2

(c) (i) The currently specified value is displayed. (The default value is 1.)
A value ranging from 1 to 8 can be entered using the Numeric keys.

(ii) The currently specified background is displayed. (The default value is 0.)
A value ranging from 0 to 8 can be entered using the Numeric keys.
The background spectrum is used to compensate for fluorescence due to the solvent and/or the flow cell. To ensure that an appropriately corrected spectrum is used in analytical work, a new background spectrum should be collected whenever the solvent or wavelength range is changed.

Input Value	**Meaning**
0	The spectrum selected by RECORD SPECTRUM No. is presented.
1 to 8	The specified background is subtracted from the spectrum selected by RECORD SPECTRUM No., and the resultant spectral data is presented.

3-3 Operation

Input

After the parameters are entered, the monitor screen presents;

```
RCD NO.     SPEED     WL-RANGE      MODE:  Em
      o        60      200-550      PRESS "START"
```

↓

START
STOP key

↓

During collection of the spectrum, the monitor screen will present:
Output monitor screen

```
RCD NO.     Ex     Em        FL       SPEED    MODE
      o    ooo    ooo    oooo.o       60         Em
```

To cancel collection of the spectrum before it is presented, press the ESCAPE key. To cancel it during output of spectral data, press the START STOP key.

Explanation

When the detector is connected to the Model D-7500 via the D-line communications network, spectral data can be stored in the Model D-7500 by pressing the ALL START key on the D-7500 instead of the START/STOP key on the detector.

NOTICE: When the ALL START key of Model D-7500 is pressed, the start signal is issued to all instruments connected through the D-line. Before pressing the ALL START key, make sure that this will not adversely affect any unit on the network.

NOTICE 1: In a fluorescence spectrum, scattered excitation radiation, Raman scattering, fluorescence due to solvent or impurities contained in the solvent, and second order (and third order) emission will be superimposed on the fluorescence of the analyte. Similarly, an excitation spectrum may be affected by scattered radiation at the fluorescence (emission) wavelength, Raman scattering, fluorescence due to solvent or impurities contained in it, and second order (and third order) scattered light of excitation beam. When selecting the optimum excitation and fluorescence wavelengths, make certain that these potential interferences are considered. For details, refer to Section 5.

NOTICE 2: The fluorescence spectrum presented by the L-7485 is not compensated for the wavelength dependent sensitivity of the photomultiplier or the gratings; thus an excitation spectrum/fluorescence spectrum attained from the L-7485 will not directly correspond to the true spectrum of the analyte. This difference will be most noticeable at emission wavelengths below 300 nm.

(12) Selecting the Confidence Function

CONFI-DENCE (⑧ Confidence)

Input

(A) Access the confidence function setup screen. These functions are used to enter information about a variety of security functions and performance related issues.

CONFI-DENCE key

(B) Select the confidence function item to be edited.

```
CONFIDENCE:  KEY LOCK=1, CHECK=2,
<1-4>  1 LOGBOOK=3, LAMP CHANGE=4
```

Press the desired Numeric key and press ENTER key.

(a) The key-lock function can be used to deactivate the keypad.

```
KEY LOCK (NO=0, YES=1)
0
```

Numeric key → ENTER key

To cancel key-lock, press the ESCAPE key. If an error is observed by the system, the keypad is automatically unlocked.

(b) The check function is used to determine the energy of the lamp and the accuracy of the monochromators. When the function is selected, the display initially presents a progress screen:

```
CHECKING LAMP ENERGY AND WL ACCURACY
```

↓

If the check function determines that the system is operating in an acceptable fashion, the display will present:

```
Ex ENERGY      Ex ACCURACY      Em ACCURACY
     oooo      GOOD  ±o nm        GOOD  ±o nm
```

If the check function determines that the system is not operating in an acceptable fashion, the display will present:

```
Ex ENERGY      Ex ACCURACY      Em ACCURACY
     oooo      FAIL  ±oo nm      FAIL  ±oo nm
```

Explanation

(A) To access the confidence function setup screen, press the CONFI-DENCE key.

Confidence function items:
- (a) Key locking/unlocking
- (b) Check of lamp energy and wavelength accuracy
- (c) Display of Xe lamp logbook data
- (d) Resetting of log book function

(B) When the screen is displayed the keylock function (1) is active. To edit this function, press ENTER . To edit another function press the apporopriate number and press ENTER .
To return to the Monitor Screen, press ESCAPE .

NOTICE: When Check is used, place ion exchange water in the flow cell, and set the holmium oxide filter (HY-1) on the Ex side of filter holder and the beam attenuator filter (ND-03, option) on the Em side.

Input Value	Meaning
1	KEY LOCK Enter '1' to lock the operation panel keys.
2	CHECK Enter '2' to check the Xe lamp energy and wavelength accuracy.
3	LOGBOOK Enter '3' to display the Xe lamp logbook data.
4	LAMP CHANGE Enter '4' to reset the logbook function.

(a) When the keylock command is selected, the currently specified value is displayed. (The default value is 0.)

Input Value	Meaning
0	The monitor screen is presented with all keys active.
1	The monitor screen is presented with all keys locked except for the ESCAPE key.

(b) Wavelength calibration is performed using the Holmium Oxide Filter (452 nm). When the Check command is selected, the data will be presented on the display for approximately 1 minute. At the end of that period, the monitor screen will be presented again.

If you want to return to the monitor screen immediately (i.e. abort the calibration), press the ESCAPE key.

Input

(c) The logbook function is used to indicate when the lamp was last changed; the display will present number of hours of use, the number of times the light was turned on, and the date that the lamp was last changed, as shown below:

```
LIGHT-ON     SWITCHING     LAST CHANGED DATE
  ooooh      oooottimes             1  1  1
```

(d) The Lamp Change function is used to reset the logbook functions when the lamp is changed. When Lamp Change is selected, the display presents:

```
LAMP CHANGE (MM DD YY)
_1 1 1
```

The date is entered in the format MM DD.

Numeral (month) (1 to 12) → ENTER → Numeral (day) (1 to 31) → ENTER

Numeral (year) (0 to 99) → ENTER

Explanation

(c) When the Logbook command is selected, the amount of time that the lamp has been used, the number of times the lamp has been turned on and the date of last change of lamp are displayed.

(d) When the Lamp Change command is selected, the date that was entered when the lamp was last changed will be presented and the cursor will appear on the first character of the month field.

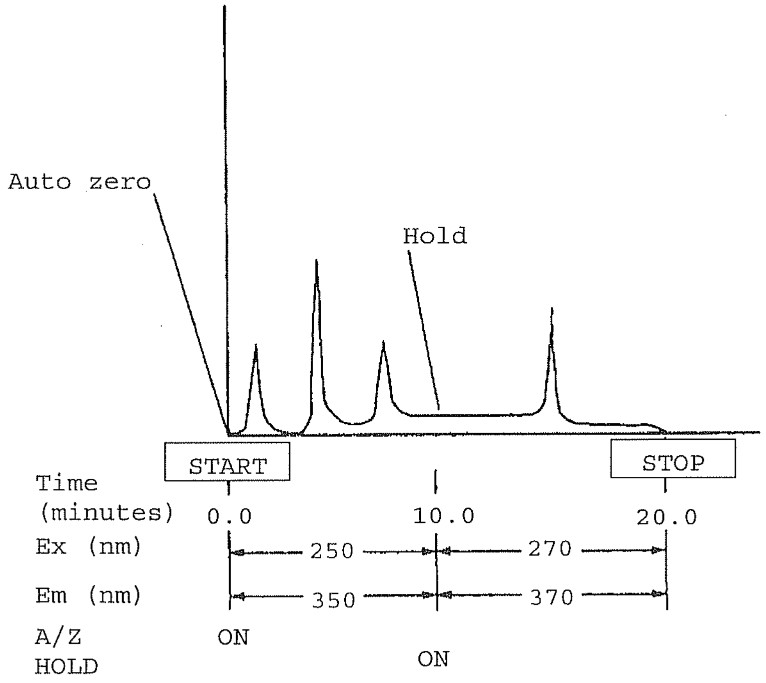
Enter the appropriate data.

NOTICE: The logbook data entry is reset when the numeric value representing 'year' and ENTER are selected. It is necessary to enter the year, even if the lamp was last changed during the present calendar year. As an example, if the lamp was changed on 3-11-94 and it is being changed on 11-27-94, make certain that 94 is reentered before ENTER is selected.

(13) Example of Time Program Operation

This selection describes how a time based program operates. The events indicated in the figure below will be used in the discussion.

(a) Setting up a Time Program


Auto zero
Hold
START
STOP
Time
(minutes)
0.0
10.0
20.0
Ex (nm)
250
270
Em (nm)
350
370
A/Z
ON
HOLD
ON

In this example, a new time program for the activity described in the above figure will be generated.

1) At the start of the separation, the Ex monochromator is set to 250 nm, the Em monochromator is set to 350 nm and the autozero function is used to define the baseline.
2) After 10 minutes, the Ex monochromator is set to 270 nm and the Em monochromator is set to 350 nm. When the monochromators are changed, the baseline is maintained (held).
3) After 20 minutes, the measurement of fluorescence is completed.

NOTICE 1: In time program setting, the initial 'TIME' for the first step must be 0.0. This initial value cannot be changed or deleted.
Be sure to specify the end time of measurement in the last step. The time program will be run until the end-of-measurement time specified at the last step is reached.

NOTICE 2: In time program setting, provide an interval of at least 0.3 minute between steps.

NOTICE 3: When a time program is selected, the display presents the operator with the opportunity to indicate if the present program should be modified or if a new program is to be entered.
If the 'MODIFY' setting is selected, the first line of the program is displayed for editing as described below.
If the 'NEW' setting is selected for a time program, the previous time program is deleted. The first step in the method will include the initial values indicated in the table below. This table also includes the allowable range for each parameter.

	Initial Value	**Input Range**
TIME	0.0	0.0 to 600.0
Ex	250	200 to 850, 0
Em	350	250 to 900, 0
BASE	1	1, 2, 0

[TIME PROG] key

```
TIME PROGRAM NO.    (1-9)
1
```

↓ [1] key → [ENTER] key

```
MODIFY=0, NEW=1
0
```

↓ [1] key → [ENTER] key

```
 TIME     Ex      Em      BASE (A/Z=1, HOLD=2)
_ 0.0     250     350        1
```

↓

([0] key →) [ENTER] key →
([2][5][0] key →) [ENTER] key →
([3][5][0] key →) [ENTER] key →
([1] key →) [ENTER] key →

```
 TIME     Ex      Em      BASE (A/Z=1, HOLD=2)
_
```

↓

[1][0] key → [ENTER] key →
[2][7][0] key → [ENTER] key →
[3][7][0] key → [ENTER] key →
[2] key → [ENTER] key→

```
 TIME     Ex      Em      BASE (A/Z=1, HOLD=2)
_
```

↓ ([2][0] key →) [ENTER] key →[ESCAPE] key

Monitor screen

```
TIME      Ex      Em      FL     PROG     LAMP     [I]
 0.0      250     350     0.0      1       Xe     (D1)
```

To indicate that the program should be stopped at a certain time, open a new step that is identical to the immediately previous step, except that the time is different. In this example, the line for 10 min (Ex 270 nm, Em 370 nm, Hold), should be repeated using a time of 20.0 min.

(b) To view a time program (e.g. to check the program that was just entered):

Press the TIME PROG key to access the Time Program screen:

↓

```
TIME PROGRAM NO.    (1-9)
1
```

↓ Enter the desired program number and press the ENTER key.
The display will present:

```
MODIFY=0, NEW=1
0
```

↓ Enter 0 and press the ENTER key.
The display will present:

```
 TIME     Ex       Em       BASE(A/Z=1, HOLD=2)
_ 0.0    250      350          1
```

↓ To access the next line, press the ∇ key.

```
 TIME     Ex       Em       BASE(A/Z=1, HOLD=2)
_10.0    270      370          2
```

↓ To access the next line, press the ∇ key.

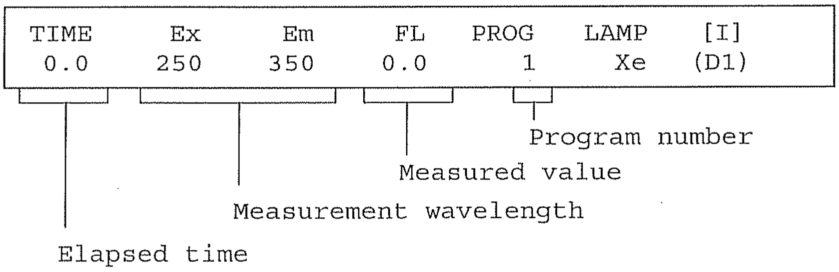
```
 TIME     Ex       Em       BASE(A/Z=1, HOLD=2)
_20.0    270      370
```

↓ To return to the first line of the program press the ESCAPE key.

```
TIME     Ex       Em       FL     PROG    LAMP    [I]
 0.0    250      350      0.0       1      Xe    (D1)
```

(c) To execute the Time Program

To execute the time program, press the START STOP key.



```
TIME     Ex       Em       FL     PROG    LAMP    [I]
 0.0    250      350      0.0       1      Xe    (D1)
```

On completion of the time program, measurement is automatically terminated and the display returns to the monitor screen. At this point, the wavelength settings are those that were on the monitor screen before the time program was executed.

NOTICE: It is advisable to set the wavelengths on the monitor screen to the same wavelengths as indicated in the Time = 0.0 of the time program.

3-4 Shutting Down the Detector

To turn the detector off, turn off the POWER switch.

The set measurement conditions are retained by the memory when the power is turned off. When the power is turned on again, the conditions that were in place when the power was turned off will be restored.

3-5 Flushing the Flow Cell After Use

Be sure to flush the flow cell with distilled water after using any buffers or salts in the mobile phase. Failure to remove buffer or salt solutions may lead to corrosion and or the formation of salt deposits in the system.

4. COMMUNICATION AND EXTERNAL INPUT/ OUTPUT SIGNALS

4-1 Communication Function Using the D-line System

The D-line system is Hitachi's unique communication network for automating a liquid chromatography system. An automated liquid chromatography system can be easily set up by connecting pumps, detectors and other devices to a data processor or integrator via the D-line network. The D-line is provided with digital communication functions and contact communication functions, either of which is selectable via the SET UP function.

4-1-1 D-line Communication Functions

The following functions are included in the D-line communication.

Table 4-1 D-line Communication Functions

	Designation	Input/ Output	Function	Signal Condition	Remarks
D-line ON	D-line (Digital line)	Input/ output	Sends the acquisition signal. Sends the acquired spectral data. Sends/receives the instrument condition parameters.	Digital	The fluorescence (FL) intensity is output.
D-line OFF	START IN (Contact input)	Input	Used to start the time program.	The time program is started when the terminals are shorted for at least one second.	
	STOP(ERROR) IN (Contact input)	Input	Used to recognize an error in other instruments on the D-line.	An error is recognized when this signal is turned on.	The lamp will be turned off when an error is recognized.
	STOP(ERROR) OUT (Contact output)	Output	Indicates an error state.	This signal remains on while there is the cause of error.	
	READY/ BUSY OUT (Contact output)	Output	Indicates the status.	This signal is turned on while the busy condition persists.	The busy condition is indicated while each function is active or in a wait state.

4-1-2 Establishing D-line Communication

To switch between the D-line function (digital line) and the contact input/output function, use the following procedure.
To use the digital communication, turn on 'D-LINE' on the D-line setup screen.

(1) Turn on the POWER switch while holding down the UTILITY key.

POWER + UTILITY

(2) When the system is first powered up, the initialization screen appears.

```
FL DETECTOR    8107064-00
Copyright (C)  Hitachi, Ltd.  2000
```

(3) After a short interval, the D-line setting screen to enable digital communication and indicate the desired channel appears.

```
D-LINE: OFF=0, ON(CH1) =1, ON(CH2) =2
        0
```

OFF : The contact input/output function is to be used.
ON(CH1) : D-line communication through channel 1 is to be used.
ON(CH2) : D-line communication through channel 2 is to be used.

* To indicate the desired option, select the appropriate value and press ENTER .

(4) To activate the D-line communication option, turn the power off and then it on again.

POWER OFF - POWER ON

4-1-3 Communication with an External Device

(1) Via the D-line Communication System

The following three digital signals are available for input/output.

(a)	Fluorescence intensity :	The fluorescence intensity at the currently set wavelength is output.
(b)	Spectra :	Acquired spectra are transmitted.
(c)	Instrument parameters :	Instrument parameters are transmitted/received. To pint the instrument parameters onto the D-7500 Integrator processor, use the UTILITY key of the Model L-7485 Detector.

(2) Contact Signal Input

A contact signal from an external switch or relay is used to control the system. Either of the signals is activated when the terminals are shorted.

(a) START IN
A contact signal input that starts the time program.

(b) STOP (ERROR) IN
A contact signal used to identify an error in another instrument that is connected to the D-line. The lamp will be turned off when the system has determined that an error has occurred if the setting for lamp off was made via the LAMP UTIL entry of the UTILITY screen.

(3) Contact Signal Output

A contact signal output for an external instrument that is used for control.

(a) STOP (ERROR) OUT
Contact signal output that indicates that an error has occurred. This signal can shut off another instrument that is connected to the system.

(b) READY/BUSY OUT
Contact signal output that indicates the instrument status. Busy status occurs when each function is in operation or in a standby state. This signal can be used to put the D-7500 in the ready status for startup.

4-1-4 Transmitting Parameters to the D-7500

The L-7485 can output instrument parameters (measurement conditions, time programs) and logbook data to the printer of D-7500 Integrator.

Table 4-2 Printout Items

Selection	Items
PARAMETER **(Instrument parameters)**	• Excitation wavelength • Fluorescence wavelength • Time constant • Photomultiplier voltage • Setting of use/non-use of time program • Lamp mode • Offset • Scan mode • Excitation wavelength scanning range • Fluorescence wavelength scanning range • Recorder full scale range • Recorder output speed • Time program
LOGBOOK	• Program number • Lamp energy • Excitation wavelength accuracy • Fluorescence wavelength accuracy • Cumulative time of lamp usage • Lamp turn-on count • Date of last change of lamp

Specify D-line output to the D-7500. For obtain a printout, use the UTILITY key.

UTILITY

```
UTILITY:  OFFSET=1,      PRINT=2,
<1-4> _1  LAMP UTIL=3,  DISPLAY MODE=4
```

Select 2 and press ENTER .
The display will present:

```
PRINT OUT (PARAMETER=1,  LOGBOOK=2)
0
```

PARAMETER : The instrument parameters are printed out.
LOGBOOK : The logbook data is printed out.

(1) Printout of Instrument Parameters

To obtain a printout of instrument parameters, indicate 1 and press ENTER .
A typical printout is shown below:

```
FL DETECTOR SETTING PARAMETERS (CH1)
Ex WAVELENGTH (nm) = 250     ← Excitation wavelength
Em WAVELENGTH (nm) = 350     ← Fluorescence wavelength
TIME CONSTANT (s)  = 2.0     ← Time constant
PMT VOLTAGE        = MEDIUM  ← Photomultiplier voltage
TIME PROGRAM       = YES     ← Setting of use/non-use of time program
LAMP MODE          = ON      ← Lamp mode

OFFSET                      = 0        ← Offset
SCAN MODE                   = Em       ← Scan mode
Ex SCANNING RANGE (nm)      = 200-850 ← Excitation wavelength scanning range
Em SCANNING RANGE (nm)      = 250-900 ← Fluorescence wavelength scanning range

RECORDER RANGE              = 1000     ← Recorder full scale range
SPECTRUM RECORD (nm/min)    = 60       ← Recorder output speed

TIME PROGRAM NO.            = 1        ← Time program number    ┐
                                                                │
TIME     Ex     Em                 ┐                            │
(min)    (nm)   (nm)      BASE     │                            │
  0.0    250    350                │   ← Contents of program    ┘
  0.5                     A/Z      │
 600.0                    HOLD     ┘
```

NOTICE: The time program will be printed out only if yes is specified for 'USE TIME PROGRAM'.

Fig. 4-1 Example of Parameter Printout

(2) Printout of Logbook Parameters

To obtain a printout of Logbook parameters, indicate 2 and press ENTER .
A typical printout is shown below.

```
FL DETECTOR LOGBOOK

                                         Depending on the instrument.

 PROGRAM NO.                 = 8108100-oo   ← Program number
 Ex LAMP ENERGY     (400 nm) = 325          ← Lamp energy
 Ex WL DIFFERENCE   (452 nm) = -oo          ← Excitation wavelength accuracy
 Em WL DIFFERENCE   (452 nm) = -oo          ← Fluorescence wavelength accuracy
 Ex LAMP LIGHT-ON TIME   (h) = 284          ← Cumulative time of lamp usage
 NO. OF LAMP SWITCHING       = 1000         ← Lamp turn-on count
 (DATE:MM/DD/YY)             = 11/12/94     ← Date of last change of lamp

If the wavelength accuracy check indicates that the setting is not correct
or if the wavelength accuracy check has not been performed, '**' is
indicated for 'WL DIFFERENCE' rather than a numerical value.
```

Fig. 4-2 Example of Logbook Printout

4-1-5 Starting and Stopping the Instrument by the D-line Communication Link

The time program stored in the L-7485 can be started by pressing the ALL START key of the D-7500. Where the autosampler is connected, the time program can be started using the START key of the autosampler.
If the xenon lamp fails or an error occurs during measurement, an error signal is issued from the L-7485 to the D-7500 Integrator through the D-line communication system. The D-7500 is required to print out the instrument parameters and logbook data of L-7485 fluorescence detector.

4-1-6 Installation

The D-line cable should be connected between the D-line terminals on the rear panels of L-7485 and D-7500 as shown in Fig. 4-3.

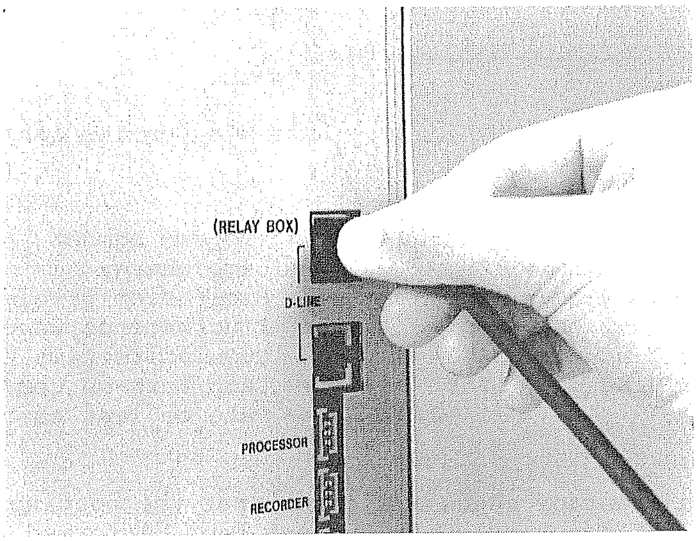


Fig. 4-3 Connecting the D-line Cable

This detector is provided with two D-line terminals, and it can be connected with the D-7500 Integrator, the L-7100 pump and L-7200 autosampler. D-line cabling, makes it possible to transmit system control signals and detector signals to each unit as shown in Fig. 4-4.

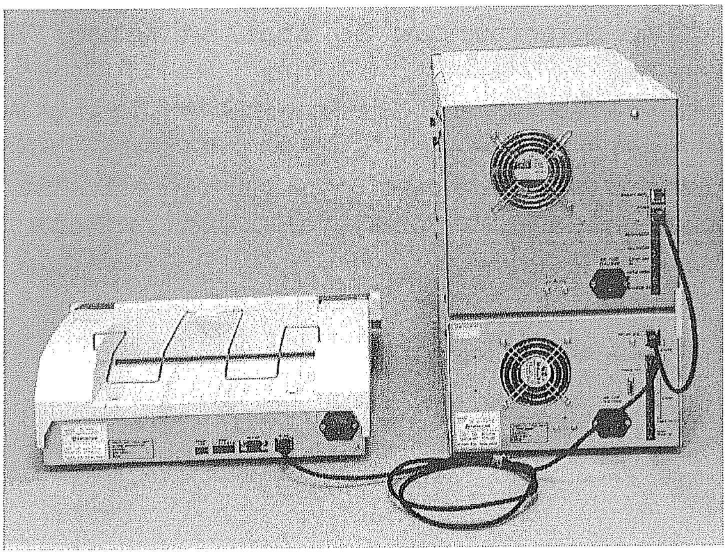


Fig. 4-4 Example of D-line Connection

4-2 Analog Communication

The L-7485 is equipped with analog input/output terminals in addition to the D-line terminals.

4-2-1 Analog Communication Functions

Table 4-3 lists the analog input/output terminals.

Table 4-3 Analog Input/output Terminals

Designation	Input/ Output	Function	Description
PROCESSOR	Output	Analog output for processor	The following signals can be output according to the setting. • Fluorescence intensity at each time point • Stored spectral data
RECORDER	Output	Output for recorder	The following signals can be output according to the setting. • Fluorescence intensity at each time point • Stored spectral data
MARKER IN	Input	The marker function is turned on by the contact signal input.	The marker function is started when the terminals are shorted for at least one second.
AUTO ZERO IN	Input	The auto zero function is turned on by the contact signal input.	The auto zero function is started when the terminals are shorted for at least one second.
LAMP OFF IN	Input	The xenon lamp is turned off by the contact signal input. The following screen appears. LAMP WAS TURNED OFF BY EXTERNAL INPUT PRESS "CL" KEY TO TURN ON	The lamp is turned off when the terminals are shorted for at least one second.

4-2-2 Exchanging Input/output Signals with an External Instrument

(1) PROCESSOR

The output terminal for a data processor can output the present fluorescence intensity or the spectrum (full scale voltage of 1 V). The following two signals are selectable for output.

(a)	Fluorescence intensity :	The fluorescence intensity at the currently set wavelength is output.
(b)	Spectrum :	Spectrum is displayed in absorbance by setting at SPECT RCD of RECORD .

When a data processor is employed and the fluorescence intensity or spectra are transmitted via analog communication, the full scale signal that can be observed is indicated in Table 4-4.

Table 4-4 ATT Setting of Data Processor and Fluorescence Full Scales

D-7500 ATT	Full Scale Voltage (mV/FS)	Fluorescence Full Scale
0	1	1
1	2	2
2	4	4
3	8	8
4	16	16
5	32	32
6	64	64
7	128	128
8	256	256
9	512	512
10	1024	1024

(2) RECORDER

This output terminal is used for the recorder. The full scale voltage is 10 mV. The full scale fluorescence intensity can be set via the RCD RANGE from 1 to 1000 (in increments of 1) of RECORD.
Two kinds of output signals can be selected. The output value is updated every 0.1 second.

- Fluorescence intensity : The fluorescence intensity at the currently specified wavelength is output to the recorder.
- Spectrum : The spectral data is output according to SPECT RCD setting of RECORD.

(3) Contact Signal Input Terminal

This is a contact signal input terminal for controlling the contact signals sent from an external switch or relay.
Any of these signals will be activated by setting "ON" (shorting) the terminal. The terminal should be "ON" for at least 1 second.

(a) MARKER IN
This function is the same as that of the MARKER key on the keyboard. (For details, refer to section 3-3 (5).)

(b) AUTO ZERO IN
This function is the same as that of the AUTO ZERO key on the keyboard. (For details, refer to section 3-3 (4).)

(c) LAMP OFF IN
An input terminal for turning off the lamp via a contact signal.

5. DESCRIPTION OF FLUOROMETRY

5-1 Principle of Fluorometry

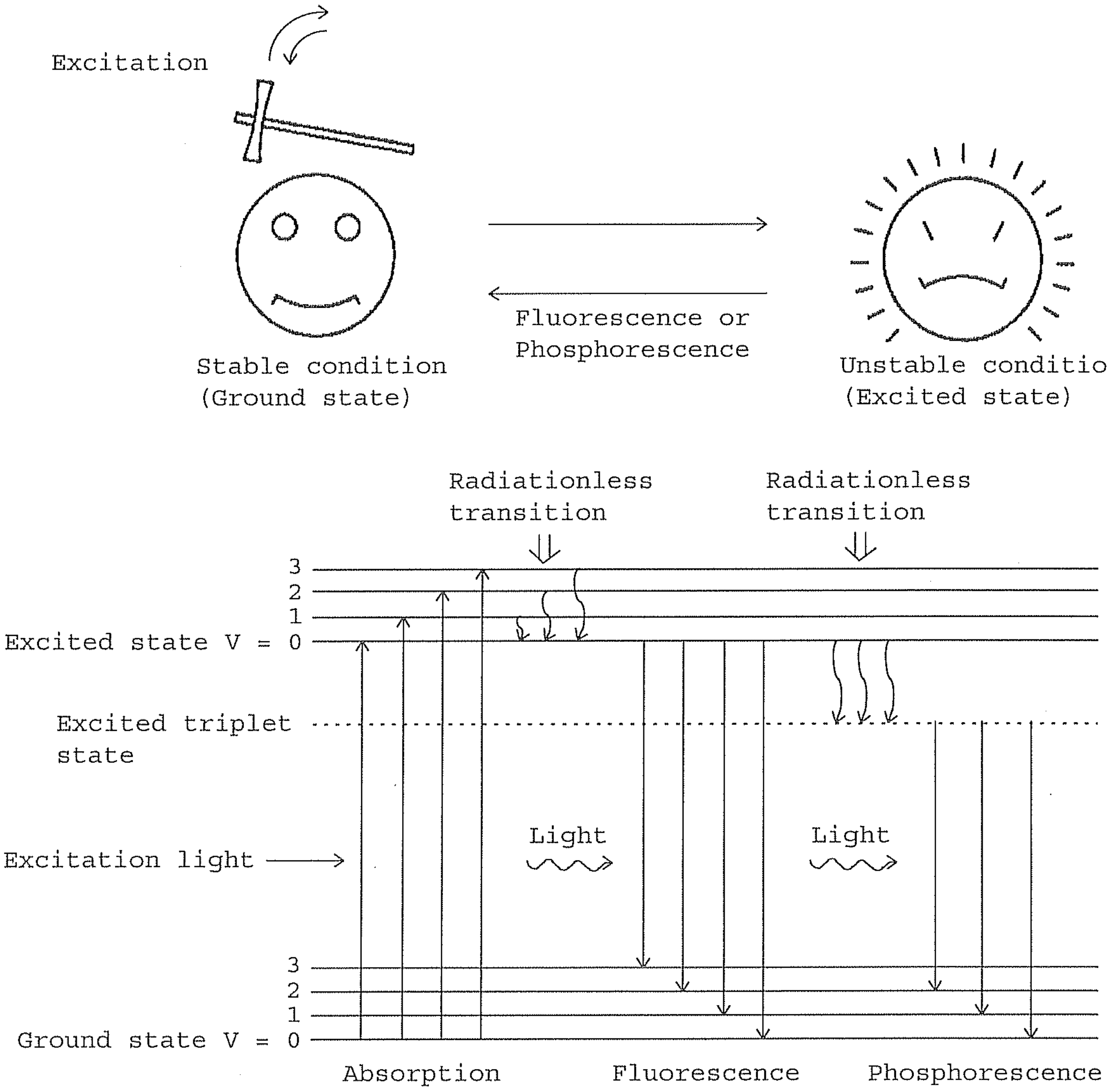

Excitation
Stable condition
(Ground state)
Fluorescence or
Phosphorescence
Unstable condition
(Excited state)
Radiationless
transition
Radiationless
transition
3
2
1
Excited state V = 0
Excited triplet
state
Excitation light
Light
Light
3
2
1
Ground state V = 0
Absorption
Fluorescence
Phosphorescence

Fig. 5-1 Typical Organic Molecular Energy Level Diagram

Figure 5-1 shows the energy level transitions of an organic molecule for the various processes involved in absorption, fluorescence and phosphorescence.

5-1 Principle of Fluorometry

When light strikes an organic molecule in the ground state, it absorbs radiation of specific wavelengths and several excited states are populated. A part of the excitation (absorbed) energy is lost in vibrational relaxation, i.e. radiationless transition to the lowest vibrational level in the excited state.

The molecule can return to the ground state by:

(1) Emitting radiation (Fluorescence)

(2) Undergoing a radiationless transition to populate the triplet state. The triplet state can emit radiation (Phosphorescence). Generally phosphorescence persists for 10^{-4} second or longer due to the selection rule imposed on the triplet-to-singlet transition. In contrast, fluorescence takes place over a period of 10^{-8} to 10^{-9} second.

(3) Going through a radiationless transition to return directly to the ground state.

Since a part of the radiation absorbed by the substance is lost as vibrational energy; the energy emitted by the excited state is less than that absorbed by the compound (i.e. the fluorescence wavelength is longer than the excitation wavelength (Stokes' law).)

The ratio of the number of photons emitted during fluorescence to the number of photons absorbed is called the quantum efficiency of fluorescence (Fluorescence Yield). If two compounds absorb the same number of photons, the fluorescence intensity of the compound with the larger fluorescence quantum yield will be greater than that from a compound with a lower fluorescence quantum yield.

When a dilute sample is used, the intensity of fluorescence is expressed by:

$$F = KI_o c l \varepsilon \phi$$

F : Fluorescence intensity
K : Instrumental constant
I_o : Intensity of exciting radiation
c : Concentration of the compound of interest
l : Optical path length of cell
ε : Absorptivity of substance
ϕ : Quantum efficiency of substance

5-2 Advantages of Fluorometry

Fluorescence can provide a significantly greater degree of sensitivity than absorption measurements. This increase in sensitivity is due to the fact that in fluorescence, the signal due to the compound of interest is measured relative to the fluorescence of the blank (which is zero). In contrast, absorbance measurements compare the transmittance of the solution of the compound of interest relative to the transmittance of the blank. As the concentration of the solution falls, the transmittance of the sample and the blank become more similar.

In this discussion, we will consider the detection of a sample that has a transmittance of 99% (relative to the blank). If we assume that the inaccuracy of the % transmittance measurement is 0.1%, the reliability of the observed data is as follows:

Percent transmittance of blank	100.0 ± 0.1%
Percent transmittance of sample	99.0 ± 0.1%
Difference (proportional to concentration of sample)	1.0 ± 0.2%

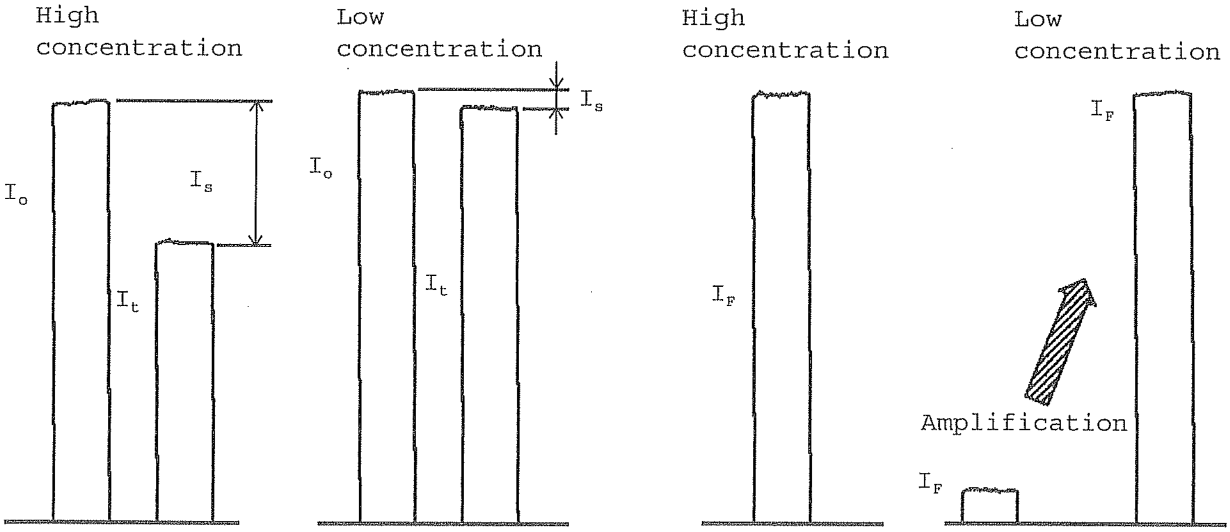
In this example, the uncertainty in the concentration measurement is ±20%. In contrast, in fluorometry, a difference in the signal is directly proportional to the concentration of sample.
An analysis of the error in the measurement is as follows:

Output signal level at measurement of sample	100 ± 0.1
Value corresponding to blank	0 ± 0.1
Difference (proportional to concentration of sample)	100 ± 0.2

The error in the measurement using fluorescence is considerably smaller than that obtained using absorbance, thus fluorometry is very advantageous for analyzing samples with a low concentration of the compound (s) of interest. If a compound exhibits a strong fluorescence signal, it is likely that fluorescence can detect concentrations that are two orders of magnitude lower than absorbance.

5-2 Advantages of Fluorometry

A graphical description of why fluorescence can provide better sensitivity than absorbance is presented in Fig. 5-2. In this figure, the signal I_s is used to represent the difference between the intensity of the incident beam I_o and the intensity of the transmitted beam I_t in absorptiometry. The detection limit is the point where the difference between I_t and I_o is equivalent to the noise level. In contrast, when fluorometry is used, the observed signal I_F is directly proportional to the concentration and the background has a fluorescence of zero. When a small signal is observed it is compared to a very small signal (since the blank does not fluoresce) and is readily amplified for detection.
In addition, since the fluorescence emission wavelength is different from the excitation wavelength (incident beam wavelength), scattering due to the excitation radiation is negligible.

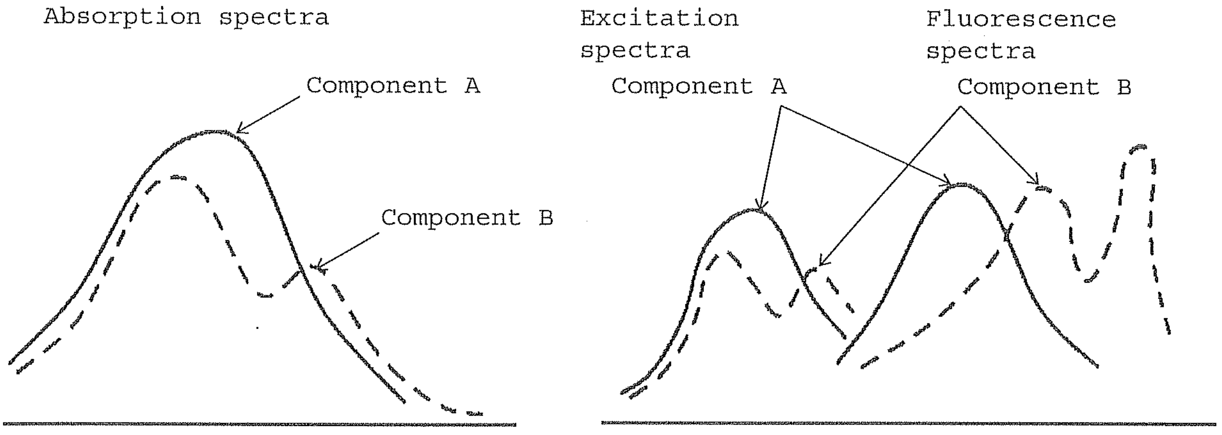


(a) Absorptiometry (b) Fluorometry

Fig. 5-2 Comparison of Absorptiometry and Fluorometry

In addition to providing high sensitivity, fluorescence detection can provide a fluorescence spectrum and an excitation spectrum (which is very similar to the absorbance spectrum). These may be useful in identifying the eluent.

If the eluent contains two compounds, selection of the appropriate excitation and emission wavelengths may be used to provide qualitative and quantitative information about the components in the mixture. This point is described in Fig. 5-3. An attempt to quantitate compound B in a mixture of A and B using absorbance will not be successful because the absorbance spectral of the two compounds overlap at all wavelengths (Fig. 5-3(a)). In contrast, if the fluorescence spectra do not overlap (Fig. 5-3(b)), quantitative analysis of each compound can be made by selecting the appropriate fluorescence emission wavelength (even if the absorption wavelengths of two components are the same).



(a) Absorptiometry (b) Fluorometry

Fig. 5-3 Spectroscopic Measurement of a Sample Containing Two Components

Table 5-1 compares the analytical information obtained via absorptiometry and fluorometry.

Table 5-1 Comparison of Analytical Information Attainable with Absorptiometry and Fluorometry

Absorptiometry	Fluorometry
Absorption spectra only (corresponding to excitation spectra)	• Excitation spectra • Fluorescence spectra

5-3 Notes on Fluorescence Analysis

5-3-1 Raman Scattering

When fluorescence is measured, two additional peaks may appear in the spectrum. The Rayleigh peak appears at the excitation wavelength and is due to scattered light, while the Raman peak appears at longer wavelength than the excitation. The position of the Raman band is dependent on the excitation wavelength, while the position of the fluorescence is independent of the excitation wavelength.

The Rayleigh peak and the Raman peak will occur even if the eluent does not contain any compound that fluoresces. If there is any doubt whether an observed peak is a Raman peak, a Rayleigh peak or a peak due to the fluorescence from the compound of interest, simply change the excitation wavelength slightly.
If the peak is due to the Raman effect or the Rayleigh effect, the observed wavelength will shift. If the peak is due to fluorescence from the compound of interest the wavelength will not change (although the peak height may change).
The Raman effect is moderately strong when water is the solvent, but is considerably weaker for other solvents that are commonly used in HPLC.

Table 5-2 presents the position of the Raman peak for a variety of excitation wavelengths.

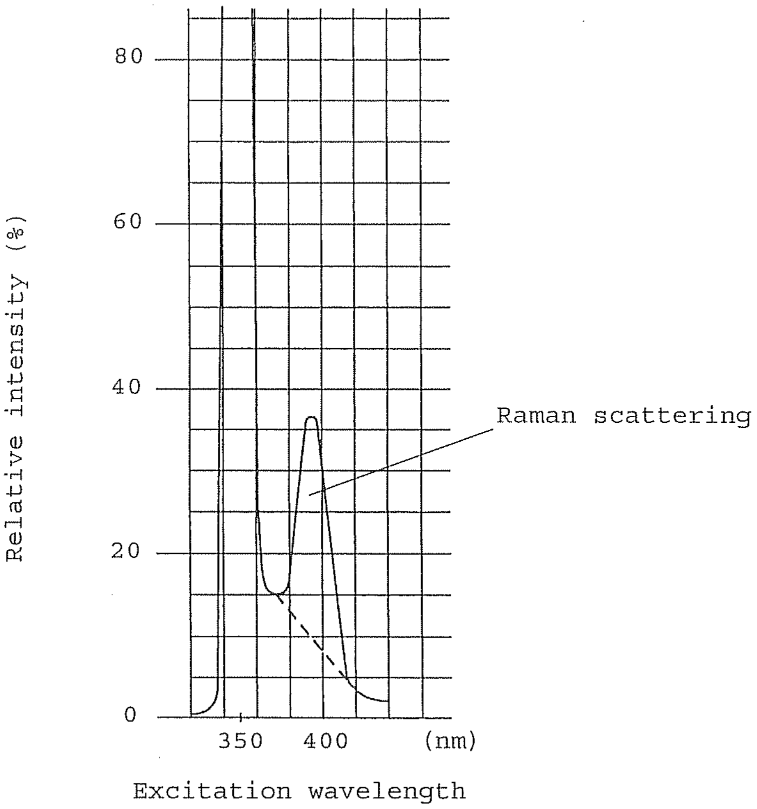


Fig. 5-4 Raman Spectrum of Water

Table 5-2 Raman Spectral Peak Position at Each Excitation Wavelength

	(Excitation Wavelength)	Water	Ethanol	Cyclohexane	Carbon Tetrachloride	Chloroform
Excitation wavelength, and Raman peak position (nm)	248	271	267	267	—	—
	313	350	344	344	320	346
	365	416	405	408	375	410
	405	469	459	458	418	461
	436	511	500	499	450	502

5-3-2 Notes on Handling Samples That Contain a High Concentration of the Compound of Interest

When the concentration of the compound of interest is relatively high, the concentration reported by fluorescence detection may be lower than the actual concentration. This phenomenon is due to the "Inner Filter effect" which is depicted in Fig. 5-5.
In this example, the concentration of the compound of interest is sufficiently high that essentially all of the excitation radiation is absorbed by molecules that are close to the entrance slit. These molecules emit, but are not in the region that is defined by the emission slit so that the fluorescence is not observed. Molecules that are in the region defined by the emission (Fluorescence) slits are not excited by the incoming radiation (since it was already absorbed).

The example described above is an extreme situation. Typically if the absorbance of the sample is greater than 0.05, the inner filter effect may reduce the observed fluorescence intensity.
To determine if the inner filter effect is reducing the fluorescence intensity, it is suggested that the sample be diluted and the intensity measured again. If the reduction in the fluorescence intensity matches the dilution, the inner filter effect is not in effect.

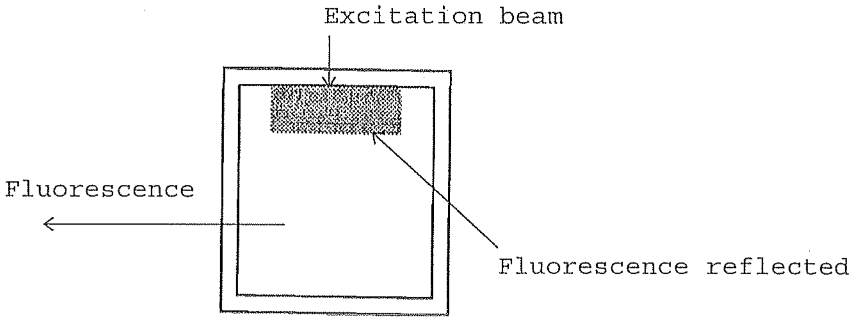


Fig. 5-5 Sample Having Extremely High Concentration

Another potential error that may occur when fluorescence detection is used is the re-absorption of fluorescence (self-absorption of fluorescence). An example of this phenomenon is shown in Fig. 5-6. Re-absorption of fluorescence occurs when the tail of the shorter-wavelength side of fluorescence spectrum overlaps the tail of the long-wavelength side of the excitation spectrum. When this phenomenon occurs, the fluorescence spectrum appears to be somewhat shifted toward the longer-wavelength.
This phenomenon will very rarely cause a substantial error in quantitation when fluorescence detection is used, and should not be a cause for concern.

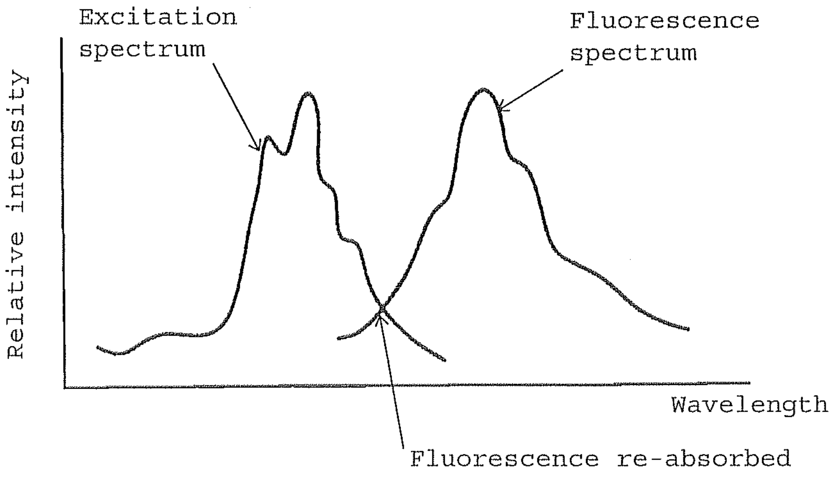


Fig. 5-6 Re-absorption of Fluorescence

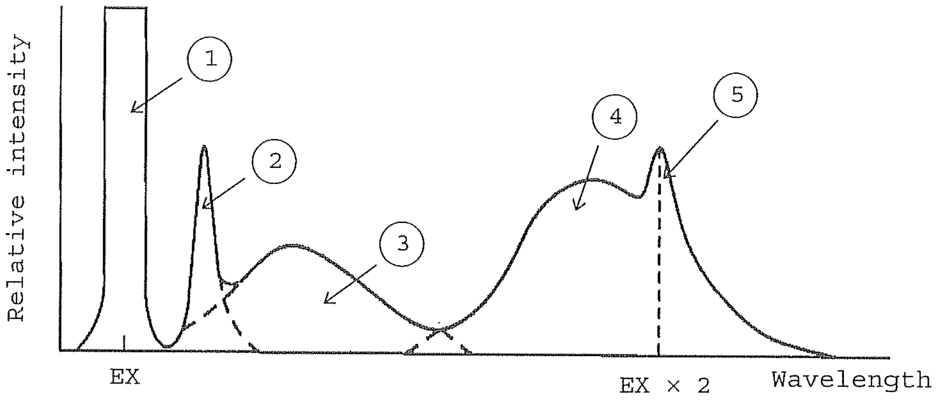
5-3-3 Second-order and Third-order Radiation

A diffraction grating that is set to transmit radiation of a specific wavelength (e.g. 250 nm) will also transmit multiples of the radiation (e.g. 500 nm). When a fluorescence spectrum is collected, the analyst should be aware of the second order (and third order) phenomena and take care that peaks are not mis-identified.

For instance, if the excitation wavelength is 240 nm, the second-order and third-order excitation occur at 480 and 720 nm, respectively. To eliminate second and third order radiation, it is simply necessary to place a short-wavelength cutoff filter in the path of fluorescing radiation (before the fluorescence monochromator).

5-3-4 An Example of a Fluorescence Spectrum

Figure 5-7 provides an example of a fluorescence spectrum which contains the various types of peaks indicated above. In most cases, the fluorescence of the compound of interest lies closer to the excitation than shown in Fig. 5-7, and overlapping of peak of interest and the second order radiation is rarely a problem. Typically, the peak for the compound of interest might correspond to peak 3, and the analyst might have to correct for the Raman peak (e.g. use a shorter excitation wavelength).



① Scattering of excitation beam
② Raman scattering due to eluent
③ Fluorescence of compound of interest
④ Fluorescence of impurity
⑤ Second-order scattering of excitation beam

Fig. 5-7 Measurement Example of Fluorescence Spectrum

5-3-5 Temperature Dependency of Fluorescence Intensity

In most cases, the fluorescence intensity decreases by 1 to 2% as the temperature of sample increases by 1 °C. It should be noted, however, that in some biochemical samples, the fluorescence intensity decreases by approx. 10% as the temperature of sample increases by 1 °C (especially if the fluorescence is due to the indole ring system).

5-4 Principle of Operation

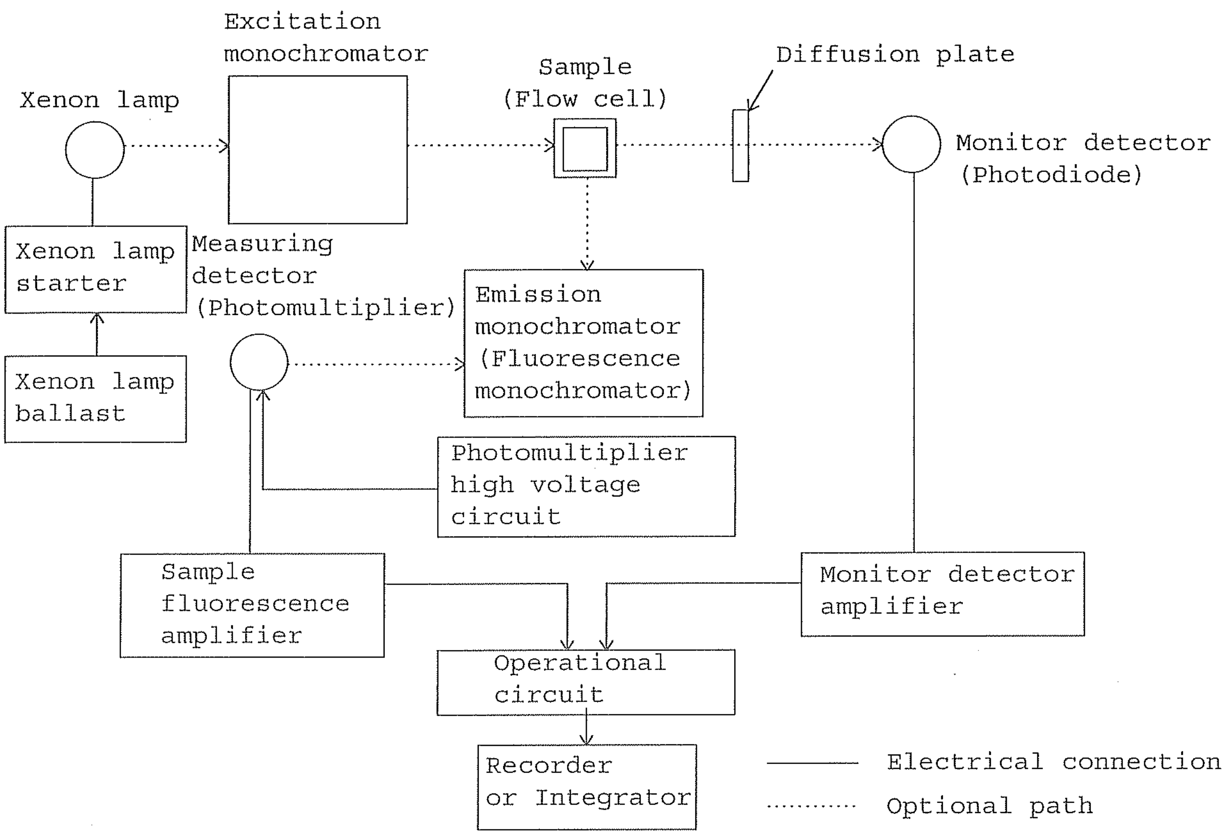

Excitation monochromator
Xenon lamp
Sample (Flow cell)
Diffusion plate
Monitor detector (Photodiode)
Xenon lamp starter
Measuring detector (Photomultiplier)
Emission monochromator (Fluorescence monochromator)
Xenon lamp ballast
Photomultiplier high voltage circuit
Sample fluorescence amplifier
Monitor detector amplifier
Operational circuit
Recorder or Integrator
Electrical connection
Optional path

Fig. 5-8 Functional Block Diagram

The beam from the xenon lamp is converged and strikes the excitation monochromator which disperses the radiation via the large-aperture concave grating. Only radiation of the specified wavelength is allowed to pass through the exit slit. After the exit slit, the excitation radiation strikes the sample.

The fluorescence (emitted radiation) enters the emission monochromator, where it is dispersed by the monochromator. The selected radiation strikes the measuring detector (photomultiplier).

The monitor detector (photodiode) measures the intensity of the excitation beam as a function of time.

5-5 Features of Light Source

The light source thermally dissociates ozone that is formed from the ultraviolet radiation via the heat generated by the xenon lamp itself. This allows the use of a fused-silica xenon lamp which produces intense radiation even in the short wavelength region.

As a safety measure against failure or stoppage of the cooling fan, a thermal sensor works to extinguish the xenon lamp in about 15 minutes if the fan stops during analysis at a room temperature of 27 °C, or in about 20 minutes if the fan was stopped from the beginning, in order to prevent failure or damage of the instrument.

6. INSTALLATION, PERFORMANCE CHECKS AND MAINTENANCE

6-1 Assembly

⚠ **WARNING**

Beware of Electric Shock!

Potentially Dangerous Voltages are Present within the Instrument.
While the POWER switch is on, a voltage of approx. 90 V is applied to the anode of xenon lamp. When the lamp goes on, a high voltage of approx. 30 kV is applied to it.
To ensure safety, however, turn off the POWER switch and unplug the power cord from the outlet.
Before removing the xenon lamp for replacement, turn power off and wait for more than two hours. Then, open the light source cover.

6-1-1 Installing the Xe Lamp

(1) Make sure the POWER switch is turned off before installing the Xe lamp. To remove the light source cover, first loosen the two mounting screws indicated in Fig. 6-1.

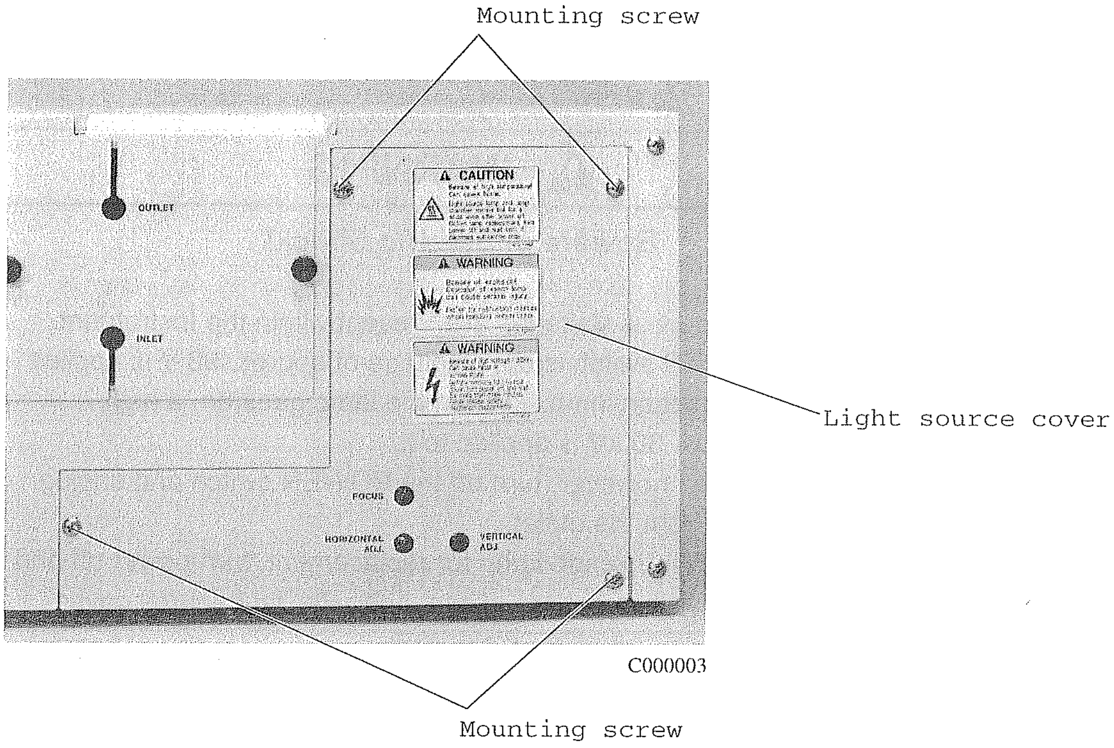

Mounting screw
CAUTION
WARNING
WARNING
OUTLET
INLET
FOCUS
HORIZONTAL ADJ.
VERTICAL ADJ.
Light source cover
C000003
Mounting screw

Fig. 6-1 Removal of Light Source Cover

(2) Wear safety goggles.

WARNING

Injury due to Lamp Explosion!

Explosion of the xenon lamp could cause serious injury. High-pressure gas at about 1 MPa is filled in the lamp at room temperature. Be careful not to apply force to the lamp bulb (glass part) when replacing the lamp. And wear protective goggles and gloves when handling the lamp.

(3) When placing the Xe lamp in the lamp holder (Fig. 6-2), put the anode terminal (marked '+') of the lamp into the metal fixture on the ceramic plate. Then, using the anode nut, mount the lamp so that the protruding part of the bulb is positioned as shown in Fig. 6-3. Do not touch the quartz envelope while the lamp is being installed.
A thin metallic wire is attached on the center area of the bulb. This wire serves to ensure that it is easily started. When handling the lamp, be careful not to break this wire. If the thin metallic wire is not located on the side of the lamp with the small quartz protrusion, side the wire so it is properly positioned.

(4) Attach the cathode lead plate to the cathode threaded part, and secure the lead plate with the cathode nut.

⚠ WARNING

Handling of Xenon Lamp

When tightening the lamp clamp nut, hold the metal parts on the anode and cathode sides with fingers. Never hold the lamp bulb when securing it. Take care not to apply excessive force or shock to the lamp bulb.
If the lamp bulb is contaminated with dust or fingerprints, wipe it using the furnished cleaning paper or gauze/absorbent cotton cloth moistened with high-quality alcohol. In this cleaning, be careful not to apply strong impact or shock to the lamp.
Be sure to mount the lamp in the specified direction. If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp.
Mount the lamp so that the '+' (anode) mark on it will be positioned at the support metal of the lamp holder.
If a lamp with its cathode consumed excessively is used continuously, the pressure inside the lamp bulb may become too high, causing explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed significantly.

Mount the lamp so that the protruding part of the bulb comes to the instrument front panel side (see Fig. 6-3).

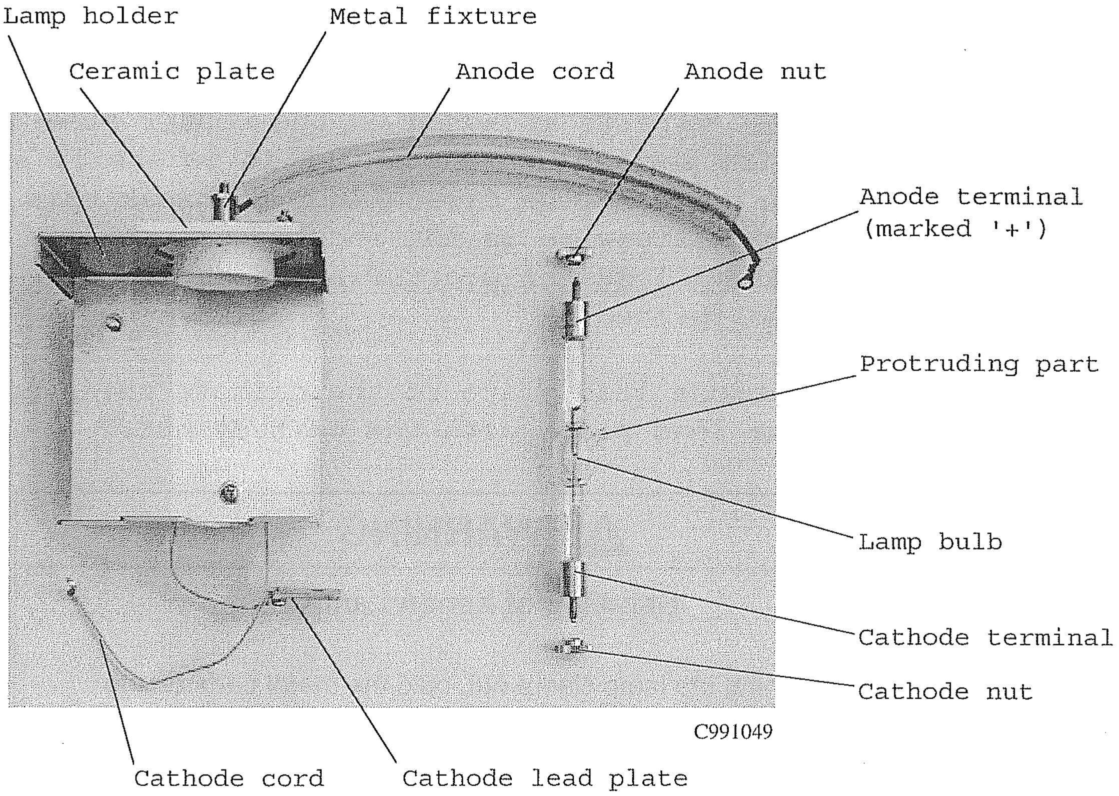

Lamp holder
Metal fixture
Ceramic plate
Anode cord
Anode nut
Anode terminal (marked '+')
Protruding part
Lamp bulb
Cathode terminal
Cathode nut
C991049
Cathode cord
Cathode lead plate

Fig. 6-2 Xenon Lamp and Its Holder

Mount the xenon lamp so that the protruding part of bulb is positioned on the same side as the anode cord.

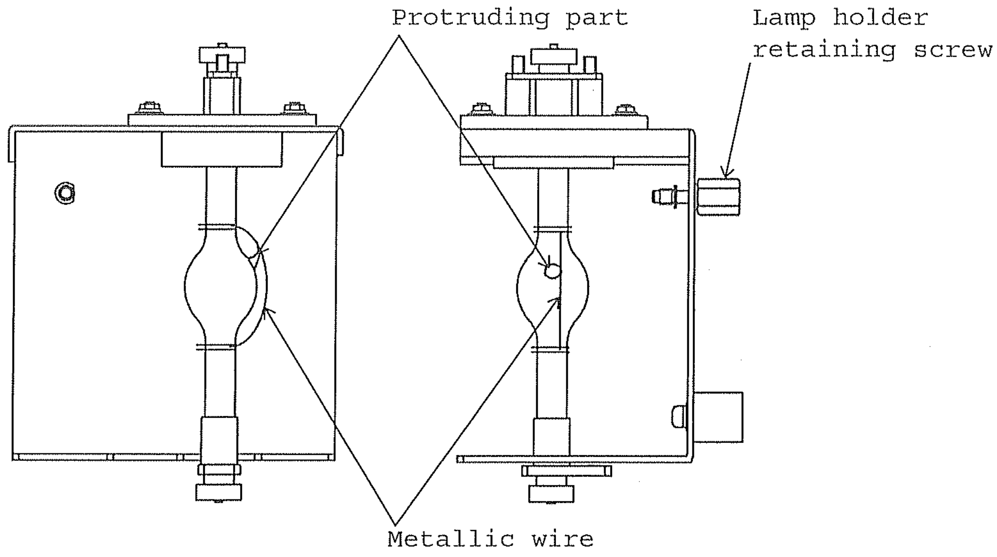

Protruding part
Lamp holder retaining screw
Metallic wire

Fig. 6-3 Protrusion on Lamp Bulb

NOTICE: Mount the xenon lamp so that the protruding part of the bulb comes to the instrument front panel side (see Fig. 6-4). A mistake in the positioning may cause an increased light loss and preclude obtaining the desired performance from the instrument.

Figure 6-4 shows how a lamp is mounted via steps (3) and (4).

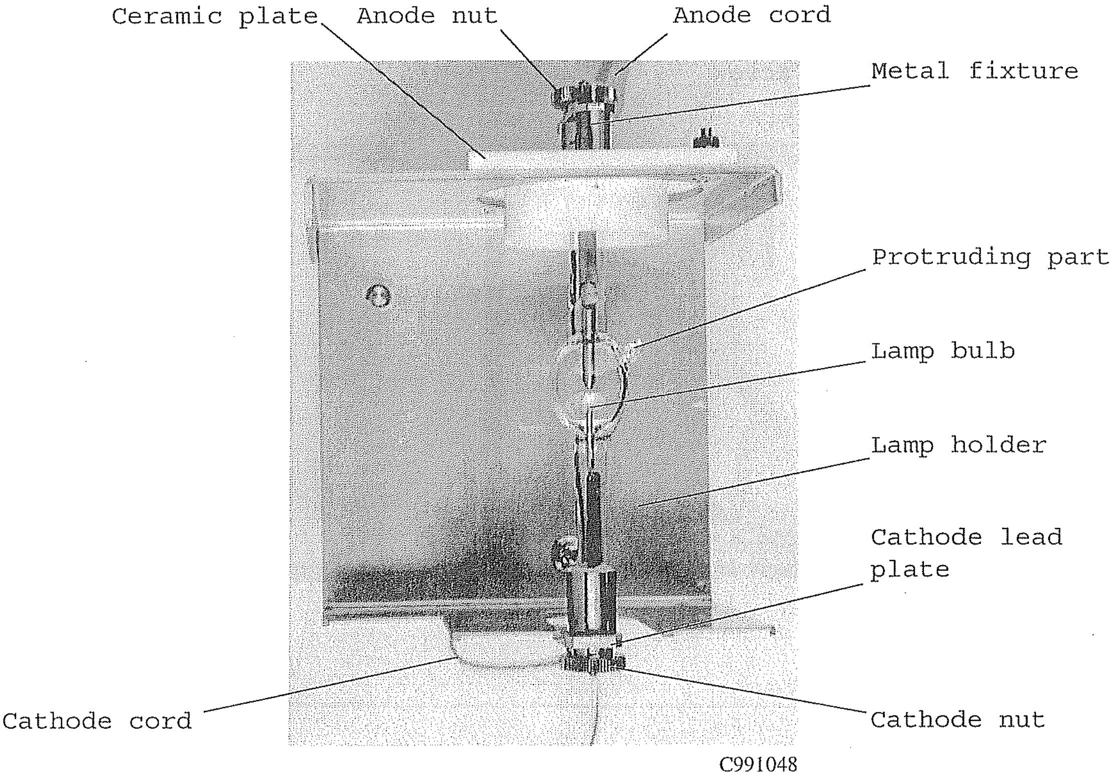

Ceramic plate
Anode nut
Anode cord
Metal fixture
Protruding part
Lamp bulb
Lamp holder
Cathode lead plate
Cathode cord
Cathode nut
C991048

Fig. 6-4 Xenon Lamp Mounted in Holder

⚠ WARNING

Explosion of Xenon Lamp

Be sure to secure the anode and cathode nuts when attaching the xenon lamp. The terminal part may heat up and explode while the lamp is lit if the nuts are loose.

(5) After mounting the xenon lamp is mounted in the holder, place the holder into the lamp housing. The holder should be secured with the lamp holder retaining screw.

(6) To connect the cables for the lamp, first loosen the connecting terminals of the lamp igniter (see Fig. 6-6). The anode cable (red) is connected to the +HV terminal of the lamp power supply and the cathode cable is connected to the COM terminal.

NOTICE: When removing the lamp holder, reverse steps (5) and (6).

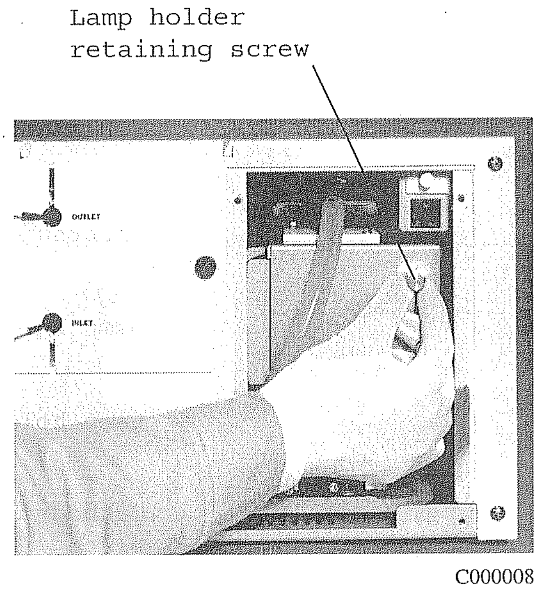

Lamp holder
retaining screw
OUTLET
INLET
C000008

Fig. 6-5 Mounting the Lamp Holder

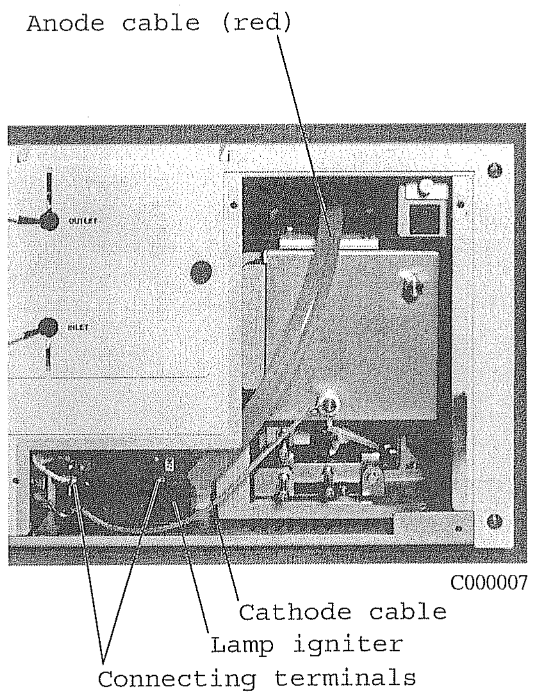

Anode cable (red)
OUTLET
INLET
C000007
Cathode cable
Lamp igniter
Connecting terminals

Fig. 6-6 Connecting the Anode and Cathode Cables

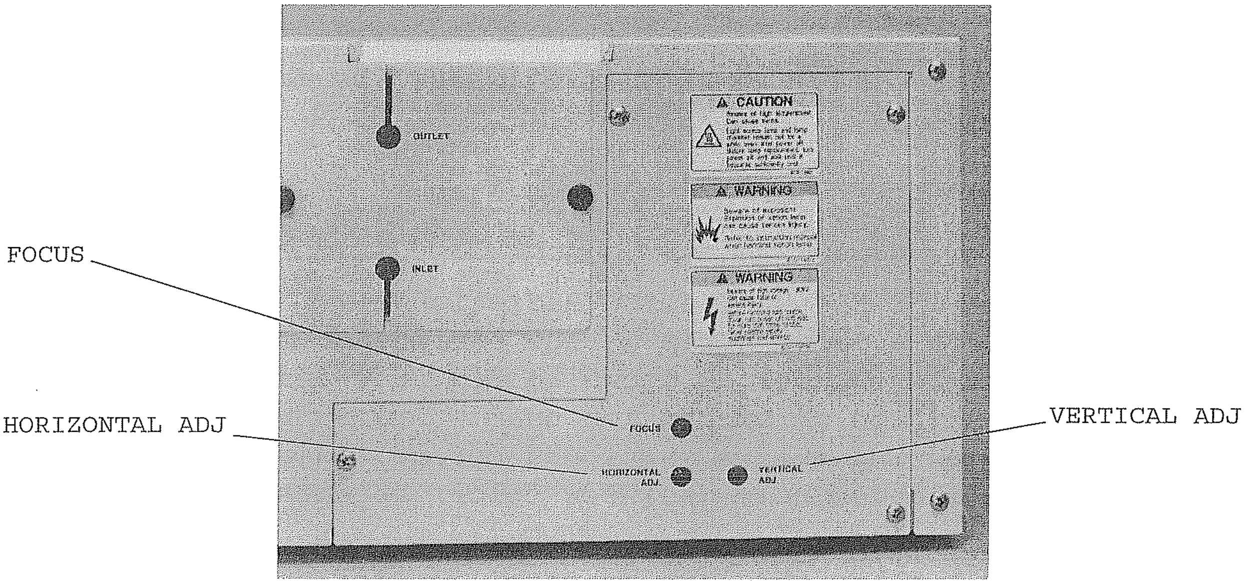
(7) After mounting the lamp holder, close the light source cover. Turn the power on and adjust the lamp as described in Section 6-1-2.

6-1-2 Adjusting the Position of Xenon Lamp

The position of the beam is slightly different from on lamp to another, so it is necessary to adjust the position of the lamp when it is first installed. This adjustment will ensure that the optimum radiation from the lamp is focused into the optical system and maximizes the sensitivity.
To adjust the lamp position, use the three adjust screws located at the right side of the instrument.

HORIZONTAL ADJ : For adjustment in the horizontal direction.
VERTICAL ADJ : For adjustment in the vertical direction.
FOCUS : For adjustment of the focal point of light source.

Adjustment of the lamp position involves a coarse adjustment (in which the position of the light image is monitored) and a fine adjustment (in which the signal detected by the monitor photomultiplier is observed). The coarse adjustment is performed first, followed by the fine adjustment.


FOCUS
HORIZONTAL ADJ
VERTICAL ADJ
OUTLET
INLET
CAUTION
WARNING
WARNING
FOCUS
HORIZONTAL ADJ.
VERTICAL ADJ.

C000003

Fig. 6-7 Adjusting the Lamp Position

(1) Coarse Adjustment

For coarse adjustment of the lamp position, use the following procedure.

(a) Turn on power to display the monitor screen.
The monitor screen will appear as shown below.

```
 TIME    Ex     Em         FL    PROG     LAMP      [I]
  0.0   250    350        0.0       1       Xe     (D1)
```

(b) Remove the flow cell unit.
The monitor screen will appear as shown below.

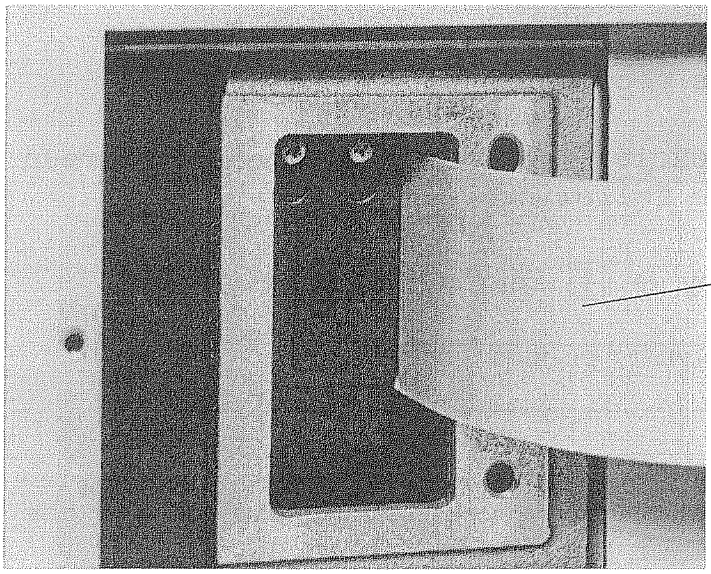
```
 TIME    Ex     Em         FL    PROG     LAMP      [I]
  0.0   250    350       ****       1       Xe     (D1)
```

(c) Set the Excitation Monochromator.
Set the excitation wavelength to 550 nm on the screen by pressing the following keys.
WAVE LENGTH key → 5 5 0 key → ENTER key →
ENTER key
After the ENTER key is pressed, the monitor will present;

```
 TIME    Ex     Em         FL    PROG     LAMP      [I]
  0.0   550    350       ****       1       Xe     (D1)
```

(d) Monitor the Excitation Radiation
Place a paper strip at the excitation beam exit of the sample chamber as shown in Fig. 6-8 and observe the condition of excitation beam. Using the three position adjusting screws mentioned before, adjust so that the excitation beam (rainbow color) becomes brightest.

NOTICE: Allow the lamp to stabilize for approximately 15 minutes after it has been turned on, before optimizing the position.



Observe excitation beam on paper

C000006

Fig. 6-8 Observation of Excitation Beam

(2) Fine Adjustment

(a) Re-mount the flow cell unit.

(b) Access the excitation energy measurement screen by pressing the following keys.

`UTILITY` key → `4` key → `ENTER` key

```
DISPLY MODE (FL=1, Ex<M>=2, Em<M>=3)
1
```

`2` key → `ENTER` key

Set the excitation wavelength to 350 nm and the photomultiplier voltage level to MED.

`WAVE LENGTH` key

```
 Ex     Em    (200-850,  0)  nm
250    350
```

`3` `5` `0` key → `ENTER` key → `ENTER` key

```
          Ex     Em    MONITOR(Ex)   LAMP       [I]
         350    350       oooo        Xe       (D1)
```

`PARAM SET` key → `ENTER` key

```
PMT VOLTAGE (HIGH=1, MED=2, LOW=3)
2
```

`2` key → `ENTER` key → `ENTER` key → `ENTER` key → `ENTER` key

```
          Ex     Em    MONITOR(Ex)   LAMP       [I]
         350    350       oooo        Xe       (D1)
```

(3) Perform the fine adjustment as described below.
Turn the FOCUS, HORIZONTAL ADJ and VERTICAL ADJ screws gradually in a repetitive manner while observing the MONITOR value on the screen. Turn each screw on an iterative basis until the MONITOR value is maximized. At least three cycles should be used. When a maximum value is reached, fine adjustment of the lamp position is completed.

After the lamp position has been optimized, access the measurement mode as described below.

UTILITY key

```
UTILITY: OFFSET=1,          PRINT=2,
<1-4> _1 LAMP UTIL=3,       DISPLAY MODE=4
```

4 key → ENTER key

```
DISPLAY MODE (FL=1, Ex<M>=2, Em<M>=3)
1
```

1 key → ENTER key
The monitor screen will the appear again.

```
TIME    Ex     Em        FL    PROG    LAMP      [I]
 0.0   350    350      ****       1      Xe     (D1)
```

6-2 Replacing the Xenon Lamp

The xenon lamp is too hot to touch immediately after the instrument is turned off. When replacing the xenon lamp with a new one, wait until the old lamp cools down completely as described below.

6-2-1 To Cool Down the Xenon Lamp

Press the following keys.

PARAM SET key → ENTER key → ENTER key → ENTER key → ENTER key

The following screen will then appear.

```
LAMP MODE  (OFF=0,  ON=1)
1
```

On this screen, press the 0 key, followed by the ENTER key to turn off the xenon lamp. The fan will continue to run to cool down the xenon lamp. Leave the power to the instrument on and wait for approximately 30 minutes.

⚠ CAUTION

Touching Hot Part Could Result in Burns!

The xenon lamp remains hot for a while even after power-off and can severely burn you if touched.
Although the xenon lamp will be automatically extinguished by a safety mechanism when the light source cover is opened, before replacement of the lamp, turn power off and wait for about 2 hours until it cools down sufficiently.

⚠ WARNING

Beware of Electric Shock!

Potentially Dangerous Voltages are Present within the Instrument.
While the POWER switch is on, a voltage of approx. 90 V is applied to the anode of xenon lamp. When the lamp goes on, a high voltage of approx. 30 kV is applied to it.
To ensure safety, turn off the POWER switch and unplug the power cord from the outlet.
Before removing the xenon lamp for replacement, turn power off and wait for more than two hours. Then, open the light source cover.

⚠ WARNING

Injury due to Lamp Explosion!

Explosion of the xenon lamp could cause serious injury.
High-pressure gas at about 1 MPa is filled in the lamp at room temperature. Be careful not to apply force to the lamp bulb (glass part) when replacing the lamp. And wear protective goggles and gloves when handling the lamp.

⚠ WARNING

Handling of Xenon Lamp

When tightening the lamp clamp nut, hold the metal parts on the anode and cathode sides with fingers. Never hold the lamp bulb when securing it. Take care not to apply excessive force or shock to the lamp bulb.

If the lamp bulb is contaminated with dust or fingerprints, wipe it using the furnished cleaning paper or gauze/absorbent cotton cloth moistened with high-quality alcohol. In this cleaning, be careful not to apply strong impact or shock to the lamp.

Be sure to mount the lamp in the specified direction. If the mounting direction (polarity) is wrong, the cathode will be consumed significantly to disable turn-on of the lamp.

Mount the lamp so that the '+' (anode) mark on it will be positioned at the support metal of the lamp holder.

If a lamp with its cathode consumed excessively is used continuously, the pressure inside the lamp bulb may become too high, causing explosion. To prevent this, replace the lamp with a new one immediately if its cathode has been consumed significantly.

⚠ CAUTION

Gazing at Illuminated Xenon Lamp Could Cause Eye Damage!

The xenon lamp radiates intense ultraviolet light when it is on. Do not look at the xenon lamp directly when it is lit. Be sure to wear tinted safety glasses to prevent possible eye damage.

6-2-2 Replacing the Xenon Lamp

(1) After the xenon lamp is cooled down as mentioned in Section 6-2-1, turn off the POWER switch. Then, remove the lamp holder (with the xenon lamp mounted) by reversing steps (1) to (6) in Section 6-1-1.

(2) Remove the xenon lamp by reversing steps (3) and (4) in Section 6-1-1.

(3) To place a new xenon lamp in the system, follow steps (1) to (6) in Section 6-1-1.

NOTICE: After the lamp has been replaced, reset the Logbook display (which indicates the date that the lamp was replaced, the number of lamp starts and the period of time that the lamp has been on). When the lamp installation date is changed, the cumulative time and the number of starts are automatically re-set to zero. For details on setting the logbook refer to Section 3-3 (12).

To set the data that the lamp was changed, use the sequence of keys indicated below (using the example of December 10, 2000). The data should be entered in the format of MM/DD/YY (e.g. 12/10/00).

CONFIDENCE key → 4 key → ENTER key → 1 key → 2 key → ENTER key → 1 key → 0 key → ENTER key → 0 key → 0 key → ENTER key

6-3 Checking the Sensitivity

6-3-1 Preparation for Sensitivity Check

The signal-to-noise ratio of the Raman spectrum of water is used to define the sensitivity of this detector. There are three steps in the overall sensitivity test:

* Filling the flowcell with water
* Collecting the Raman spectrum
* Determining the Noise at the maximum fluorescence intensity

For this measurement, the following items are required:

(1) HPLC Pump

(2) Recorder or D-7500 Integrator

(3) Water

The water should be distilled or purified in a similar fashion (e.g. reverse osmosis or passed through appropriate filters). It is suggested that the resistance of the water should be 18.3 meg or higher. HPLC grade water is acceptable for the measurement of sensitivity.

NOTICE: Water is used in several tests described in this chapter. Always use distilled water or water purified as described above for these tests.

6-3-2 Pumping Water into the Cell

Connect a tube from the outlet of the Model L-7100 Pump to the inlet of the Model L-7485 Fluorescence Detector as shown in Fig. 6-9. Set the pump to Manual mode and deliver water at a flow rate of 1 mL/minute for a period of three minutes. After the three minute period, set the flow rate to zero, remove the flow cell unit and check that it is free of air bubbles. After the flow cell has been inspected and air bubbles removed (if necessary), replace the cell in the detector.

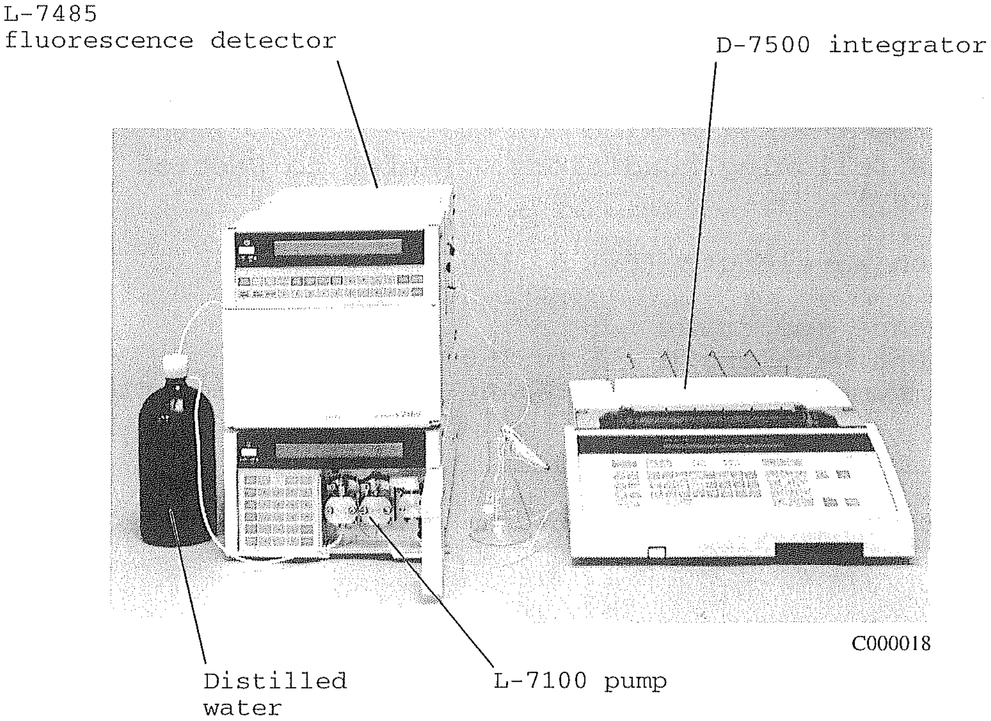

L-7485
fluorescence detector
D-7500 integrator
C000018
Distilled
water
L-7100 pump

Fig. 6-9 Tubing Connection to Place Water in the Flow Cell

Turn on power to the L-7485 detector.

NOTICE: If the flow cell has been left empty for several days (including the installation), fill it with methanol or acetonitrile before water in order to wet the flow path wall in advance.
Filling flow cell with water without the pre-wetting may preclude obtaining the proper performance from the instrument.

6-3-3 Measuring the Raman Spectrum of Water

Enter the following conditions. (Refer to Section 3-3 (10) for assistance.)

Excitation wavelength (Ex) : 350 nm
Response : 2 seconds
Fluorescence spectrum measurement wavelength : 350 to 450 nm
Spectrum number : Any unoccupied position can be used (1 to 8)

```
TIME   Ex     Em        FL    PROG    LAMP     [I]
 0.0  350    350       0.0      1      Xe     (D1)
```

WAVE LENGTH key

```
 Ex    Em    (200-850,  0)  nm
250   350
```

3 5 0 key → ENTER key → ENTER key

UTILITY key

↓

```
UTILITY: OFFSET=1,       PRINT=2,
<1-4> _1 LAMP UTIL=3,    DISPLAY MODE=4
```

↓

4 key → ENTER key

↓

```
DISPLAY MODE (FL=1, Ex<M>=2, Em<M>=3)
1
```

↓

1 key → ENTER key

↓

```
TIME   Ex     Em        FL    PROG    LAMP     [I]
0.0   350    350      ****      1      Xe     (D1)
```

↓

RECORD key

```
RECORD :    RCD RANGE=1, RCD SPEED=2,
<1-3>  1    SPECT RCD=3
```

↓

2 key → ENTER key

```
RCD SPEED (40 nm/min=1, 60 nm/min=2)
2
```

↓

2 key → ENTER key

↓

```
TIME    Ex    Em       FL    PROG    LAMP     [I]
0.0    350   350      ****      1      Xe    (D1)
```

RECORD key

↓

```
RECORD :    RCD RANGE=1, RCD SPEED=2,
<1-3>  1    SPECT RCD=3
```

↓

1 key → ENTER key

↓

```
RECORDER RANGE  (1-1000)
1000
```

↓

1 0 0 0 key → ENTER key

↓

```
TIME    Ex    Em       FL    PROG    LAMP     [I]
0.0    350   350      ****      1      Xe    (D1)
```

↓

PARAM SET key

↓

6-3 Checking the Sensitivity

```
TIME CONSTANT   (0.1s=1,  0.5s=2,  2.0s=3,
3                4.0s=4,  8.0s=5)
```

↓

$\boxed{3}$ key → $\boxed{\text{ENTER}}$ key

↓

```
PMT VOLTAGE (HIGH=1,  MED=2,  LOW=3)
1
```

↓

$\boxed{2}$ key → $\boxed{\text{ENTER}}$ key → $\boxed{\text{ENTER}}$ key → $\boxed{\text{ENTER}}$ key → $\boxed{\text{ENTER}}$ key

↓

```
TIME    Ex    Em       FL   PROG    LAMP     [I]
0.0    350   350      ****     1      Xe    (D1)
```

$\boxed{\text{SPECT PARAM}}$ key

↓

```
SCAN MODE  (Ex=1,  Em=2)
2
```

↓

$\boxed{2}$ key → $\boxed{\text{ENTER}}$ key

↓

```
Em  SCANNING WL RANGE  (250-850) nm
300  - 500                        1 nm  step
```

↓

$\boxed{3}$ $\boxed{5}$ $\boxed{0}$ key → $\boxed{\text{ENTER}}$ key

↓

```
Em  SCANNING WL RANGE  (ooo-900) nm
350  - 500                        1 nm  step
```

↓

$\boxed{4}$ $\boxed{5}$ $\boxed{0}$ key → $\boxed{\text{ENTER}}$ key

↓

```
TIME    Ex    Em       FL   PROG    LAMP     [I]
0.0    350   350      ****     1      Xe    (D1)
```

SPECT MEMO key

Scanning is initiated when the SPECT MEMO key is pressed.

↓

```
SPECTRUM MEMORY NO.  (1-8)   MODE:Em
No.  _
```

↓

X key → ENTER key

X: Memory number (1 to 8)
After the spectrum has been collected, press the memory number, to return to the monitor screen.

In this example, X=2

↓

```
SCANNING SPECTRUM NO. 2
Ex:350      Em:ooo
```

↓

```
TIME   Ex    Em      FL   PROG   LAMP    [I]
0.0   350   350    ****      1     Xe   (D1)
```

RECORD key

```
RECORD : RCD RANGE=1, RCD SPEED=2,
<1-3>  1  SPECT RCD=3
```

↓

3 key → ENTER key

```
RECORD SPECTRUM NO.(1-8)
X
```

↓

Enter the memory location number specified for SPECT MEMO.

X key → ENTER key

X: Memory number (1 to 8)

↓

```
BACKGROUND SPECTRUM NO.  (0-8)
0
```

↓

0 key → ENTER key

For example: X=2

6-3 Checking the Sensitivity

```
RCD NO.     SPEED     WL RANGE     MODE:Em
     2         60     350-450      PRESS"START"
```

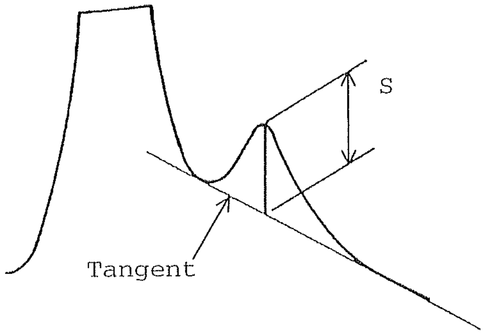
NOTICE: Set the D-7500 Integrator as indicated below.
ATT: 5
CHART SPEED: 60 mm/minute

Press the START STOP key on the detector and start the recorder or integrator by pressing the START STOP key on the recorder or integrator. These two actions initiate the collection of data.

NOTICE: Where the detector is connected with the Model D-7500 Integrator through the D-line, collection of the spectrum can be initiated by pressing the ALL START key of the Integrator.

6-3-4 Measuring the Raman Signal Intensity

A typical spectrum is presented below.



To determine the signal intensity, draw a line from the valley between the Rayleigh peak and the Raman peak to the tail of the Raman peak as shown in the above figure and measure the height of the tangent.
If the Model D-7500 Integrator is used, determine 'S' using the length on the output and the equation:

$$S = \frac{L}{152} \times 2^{X} \text{ (mV)}$$

where: L is the length of S (in mm)
X is the ATT (attenuator) setting on the Integrator.

6-3-5 Measuring the Peak Noise

Keep the excitation wavelength at 350 nm, and set the fluorescence wavelength to 397 nm. Using the WAVE LENGTH key, change the fluorescence wavelength slightly to determine the exact wavelength where the fluorescence is maximized. When the wavelength for the fluorescence intensity maximum has been determined, start the recorder tot the D-7500 Integrator to record the baseline.

NOTICE: The room temperature should be held constant during this measurement.

When using the D-7500, it is advisable to set ATT to '0'.

6-3-6 Calculating the Noise for the Raman Peak

To determine the noise (N) for the Raman peak:

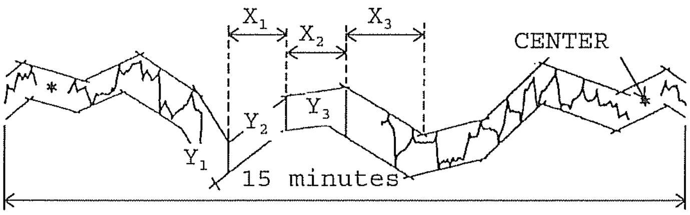
(1) Monitor the baseline for 15 minutes

(2) Draw lines that define the noise envelope for each one minute period (see Fig. 6-11).

(3) Calculate the noise (in mV) via the equation:

$$N = \frac{\text{Noise width}}{152} \times 2^{X} \quad (\text{mv})$$

where: X is the ATT (attenuator) setting on the integrator



('Xn' shall be determined for periods of one minute.)

$$\text{Noise} \cdot \text{width} = \sum_{R=1}^{R=n} Y_R / n \quad (\text{mm})$$

where, Y_R is a length (mm) on chart at each time period.

Fig. 6-10 Noise Calculation (Extracted from ASTM E685-79(1979))

NOTICE: The Raman spectrum of water and the noise level may be affected by purity of the water and/or the presence of air bubbles. If the Raman band has a very long tail, it is likely that the water is not sufficiently pure and contains fluorescent impurities. The presence of impurities will lead to an increase in the S/N ratio, while the presence of air bubbles will reduce the S/N value.

6-3-7 S/N Ratio Calculation of Raman Peak of Water

Using the Signal (S) obtained in Section 6-3-4 and the Noise (N) obtained in Section 6-3-6, calculate the signal-to-noise ratio of Raman peak of water as indicated below.

S/N ratio = S/N (Specification: S/N ratio ≥ 350)

6-3-8 Example of Measuring Raman Peak of Water Using a Time Program

When Raman signal intensity obtained in Section 6-3-3 is low or when the sensitivity (S/N ratio of Raman peak of water) is low, re-check the following items.

(1) When noise is large.
  (a) Check the distilled water *.
  (b) Check the position and useful life of xenon lamp.

(2) When peak separation is not good.
  (a) Check the distilled water *.
  (b) Change the method of spectrum measurement (via time program)

Measure the Raman peak of water using a time program. But first check the fluctuation in FL value via wavelength shift. Set the excitation wavelength to 350 nm and the fluorescence wavelength to 450 nm with the following keys. WAVE LENGTH key → 350 key → ENTER key → 450 key → ENTER key. Then zero the FL value with AUTO ZERO key. Using WAVE LENGTH key, check the wavelength where the FL value is maximum near a fluorescence wavelength of 397 nm, and the wavelength where the FL value is minimum near a fluorescence wavelength of 380 nm. The minimum FL value should be less than half the maximum FL value.

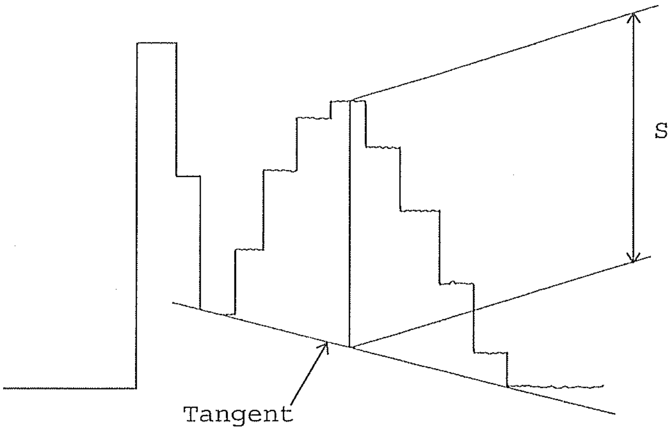
* For the distilled water, use water soon after it has been purified. If left for a long time in a container made of resin or the like, fluorescent substance may mix in with the distilled water.

Create and execute the following time program for the Raman peak of water.

TIME	Ex	Em	BASE (A/Z = 1, HOLD = 2)
0.0	350	450	1
0.5		360	
0.6		370	
0.7		375	
0.8		380	
0.9		385	
1.0		390	
1.1		395	
1.2		400	
1.3		405	
1.4		410	
1.5		415	
1.6		430	
1.8		450	
2.0			

NOTICE: The above time program is an example where the minimum FL value was at 380 nm and the maximum was at 400 nm. At the top Em of the program, set a wavelength that includes the wavelengths where maximum and minimum FL values were obtained. (For instance, if a maximum FL value was obtained at 397 nm, then the wavelength at 1.1 minutes should be set at 397 nm.)

Read out the value corresponding to "S" from the spectrum obtained with the time program.



If the Model D-7500 Integrator is used, "S" can be determined using the length on the output and the following equation:

$$S = \frac{L}{152} \times 2^{x} \qquad \text{(mV)}$$

where: L is the length of S (in mm)
X is the ATT (attenuator) setting on the Integrator.

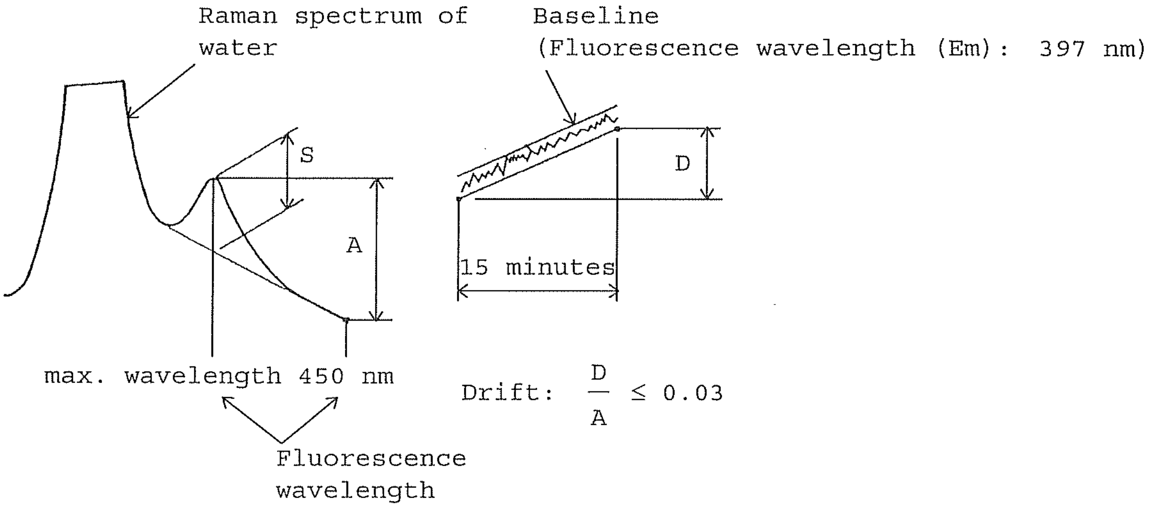
Refer to Sections 6-3-5 to 6-3-7 for noise measurement and calculation and S/N ratio calculation.

6-3-9 Drift Measurement

To measure the signal drift, use data from the Raman band used to calculate the signal to noise ratio.

The drift is defined as D/A and should be less than 0.03.

D is the change in the signal at 397 nm over a period of 15 minutes, and A is the difference in the signal between 397 and 450 nm (for the sake of simplicity, perform the calculation on the assumption that the signal is zero at the fluorescence wavelength of 450 nm). D and A are indicated in the figure below.


Raman spectrum of water
Baseline
(Fluorescence wavelength (Em): 397 nm)
S
A
D
15 minutes
max. wavelength 450 nm
Fluorescence wavelength

Drift: $\frac{D}{A} \leq 0.03$

Condition:
Excitation wavelength (Ex); 350 nm
Response; 2 seconds
Flow cell; 12 μL

NOTICE: Carry out the drift measurement after the xenon lamp becomes stable. It is suggested that the drift measurement be taken after the lamp has been on for at least one hour.

6-4 Wavelength Accuracy Check and Calibration

6-4-1 Checking Wavelength Accuracy

(1) Principle of Measurement

The wavelength accuracy measurement employs the bright line at 436 nm that is emitted by a fluorescent lamp (such as an overhead room light). The wavelength accuracy of emission monochromator is determined by checking the wavelength of the bright line. Using the emission monochromator set at the wavelength at which the bright line is detected, the wavelength accuracy of excitation monochromator is determined by checking the wavelength of excitation light which makes Raman scatter at 436 nm and comparing with theoretical value(380 nm).
The wavelength of Raman scatter is defined physically by the wavelength of excitation light and solvent.
The relation between wavelength of Raman scatter(λ_{Raman}) and that of excitation light(λ_{Ex}) is represented by the theoretical formula below.

$k_{Raman} = k_{Ex} - k_{Water}$;

k_{Raman} :Wave number of Raman scatter, $k_{Raman} = 1/\lambda_{Raman}$

k_{Ex} :Wave number of excitation light, $k_{Ex} = 1/\lambda_{Ex}$

k_{Water} :Wave number of molecular vibration of water, $3.38\text{x}10^5$ (m^{-1})

(2) Checking Fluorescence Wavelength Accuracy

(a) Remove the light sampling screw of the flow cell unit.

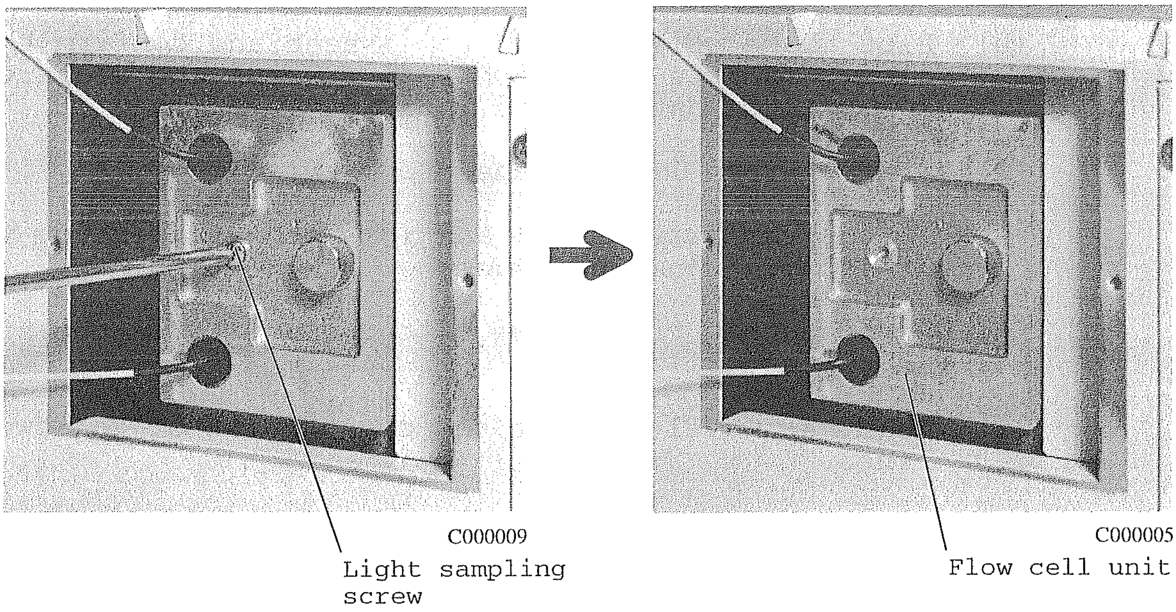


Fig. 6-11 Removal of Light Sampling Screw

(b) Using the PARAM SET key, turn off the xenon lamp.
PARAM SET key → ENTER key → ENTER key → ENTER key →
ENTER key → 0 key → ENTER key

(c) Using the PARAM SET key, set PMT voltage "LOW".
PARAM SET key → ENTER key → 3 key → ENTER key

(d) Using the PARAM SET key, set Em bandwidth "STANDARD".
PARAM SET key → ENTER key → ENTER key → 1 key
→ ENTER key

(e) Using the UTILITY key, change the DISPLAY mode to Em<M> mode.
UTILITY key → 4 key → ENTER key → 3 key →
ENTER key

(f) Using the WAVE LENGTH key, set a fluorescence wavelength to 426 nm and read the energy value.
WAVE LENGTH key → ENTER key → 4 2 6 key →
ENTER key

(g) In the same way, read the energy values at 428nm and 430nm.

(h) Plot these points on a graph sheet and confirm them in a row. If not, add another data point and acquire at least three data points in a row.

(i) Draw a trend line of these points.

(j) In the same way as (f) and (g), read the energy values at 442nm, 444nm and 446nm.

(k) Draw another trend line of these points in the same way as (h) and (i).

(l) Read the wavelength at the cross point of the two trend lines.

(m) If the wavelength is outside the specified range of 436 ± 3 nm, it is necessary to calibrate the emission monochromator as described in Section 6-4-2.

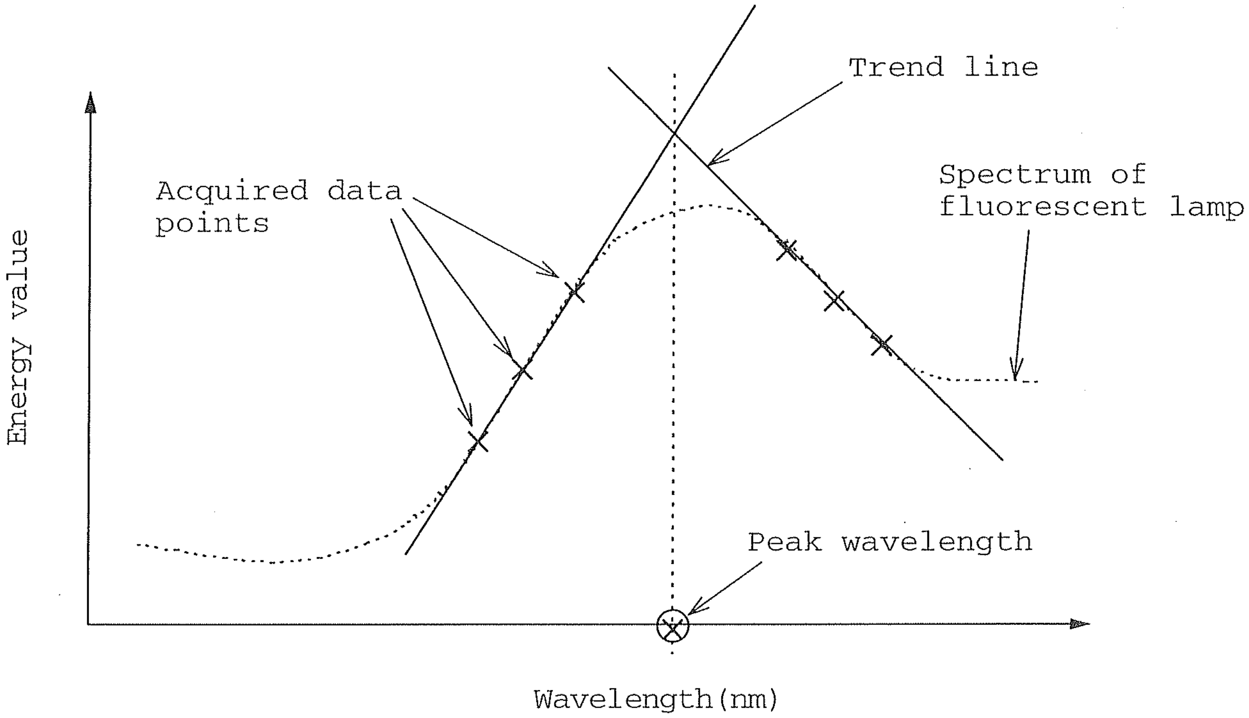


Fig. 6-11-1 Determination of Peak wavelength

NOTICE 1:	When checking the accuracy of the fluorescence monochromator, take special precautions to ensure that radiation from other sources cannot enter the instrument (e.g. do not place the unit near a window or near a source of room light).
NOTICE 2:	If you move around in front of the sample chamber during the check of the fluorescence wavelength accuracy, the observed intensity of the fluorescent lamp light may vary. This may prevent you from accurately determining the peak wavelength.
NOTICE 3:	If the energy level appears to be saturated during the determination of the wavelength accuracy, adjust the amount of light entering the monochromator by turning off some lamps in the laboratory.
NOTICE 4:	In wavelength accuracy check, set the emission monochromator bandwidth at the standard (15 nm).

(3) Checking Excitation Wavelength Accuracy

(a) Attach the light sampling screw of the flow cell unit.

(b) Set the fluorescence wavelength to the position determined at step (2)-(1).

(c) Using the PARAM SET key, turn on the xenon lamp.
PARAM SET key → ENTER key → ENTER key → ENTER key → 1 key → ENTER key

(d) Using the PARAM SET key, set PMT voltage "MED".
PARAM SET key → ENTER key → 2 key → ENTER key

(e) Using the UTILITY key, change the DISPLAY mode to Em<M> mode.
UTILITY key → 4 key → ENTER key → 3 key → ENTER key

(f) Using the WAVE LENGTH key, set the excitation wavelength to 380 nm.
WAVE LENGTH key → 3 8 0 key → ENTER key → ENTER key

(g) Using the WAVE LENGTH key, shift the excitation monochromator (Ex) so that the indicated energy value is maximized.
If the excitation wavelength is outside the specified range of 380 ± 3 nm, it is necessary to calibrate the excitation wavelength accuracy as described in Section 6-4-2.

NOTICE: Before determining the excitation wavelength accuracy, ensure that the lamp is properly focused (Section 6-1-2).

(4) End of Check

This completes the check of wavelength accuracy.

6-4-2 Correcting for Inaccuracy in the Monochromator Setting

If a significant deviation is found in the wavelength accuracy check, it is necessary to adjust the wavelength via the wavelength calibration (Special) mode.

(1) To Correct the Excitation Monochromator Setting

To access the excitation wavelength calibration mode, press the following keys.

$\boxed{\text{UTILITY}}$ key → $\boxed{9}$ $\boxed{9}$ key → $\boxed{\text{ENTER}}$ key
(Numeric value: 99)

The present offset value is indicated on the wavelength calibration mode screen. The indicated value is used as the reference value as described below.

```
Ex WL ADJUST (1-20)
 10                                        ( 2)
```

To calibration the monochromator, enter the value determined using the equation below.

$\boxed{\text{Numeric}}$ key → $\boxed{\text{ENTER}}$ key
(Numeric value: 1 to 20)

Calculation of new offset numeric value:

$$\left[\begin{array}{l}\text{Present offset}\\ \text{value}\end{array}\right] - \left[\begin{array}{l}\text{Deviation of Wavelength}\\ \left(\begin{array}{l}\text{Peak wavelength}\\ \text{value obtained in}\\ \text{Section 6-4-1}\end{array}\right) - \left(\begin{array}{l}\text{Normal peak}\\ \text{wavelength}\\ \text{value: 380 nm}\end{array}\right)\end{array}\right] = \left[\begin{array}{l}\text{New}\\ \text{offset}\\ \text{value}\end{array}\right]$$

Example: $10 - \{(383) - (380)\} = 7$
The allowable input range for the offset is from 1 to 20.
After wavelength calibration, check the wavelength accuracy again.

NOTICE: When the wavelength accuracy has been checked using the CONFIDENCE function, the wavelength deviation is indicated in parentheses at the lower right corner of the screen.

(2) For Calibration of the Emission (Fluorescence) Monochromator Wavelength

To access the emission (fluorescence) wavelength calibration mode, press the following keys.

UTILITY key → 9 8 key → ENTER key
(Numeric value: 98)

For wavelength calibration, enter the appropriate offset. The calculation is performed in the same way as described for the excitation monochromator except the normal peak wavelength is 436nm.

6-5 Checking and Cleaning the Flow Cell Unit

6-5-1 Checking and Cleaning of Flow Cell

If the inside of the flow cell is contaminated, it must be cleaned.

The following items are required for cleaning the flow cell.

NOTICE: A glass syringe with capacity of approximately 10 mL and the appropriate washing solution (see below).

(1) Loosen the clamp screw retaining the flow cell unit.

(2) Remove the flow cell.

(3) Check the inside of the cell to see if it is contaminated with any foreign substance (e.g. oils, salts or buffers).

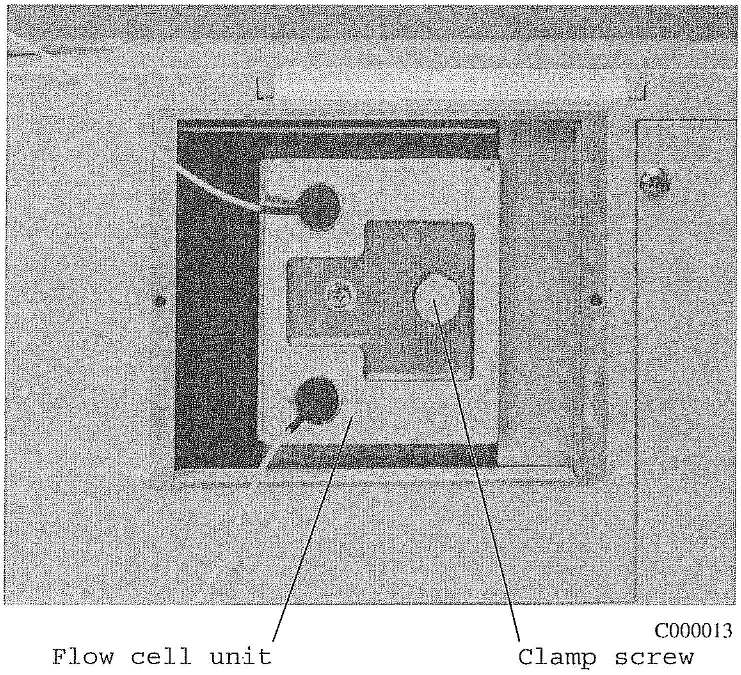
(4) Check the outside of the cell for leakage.

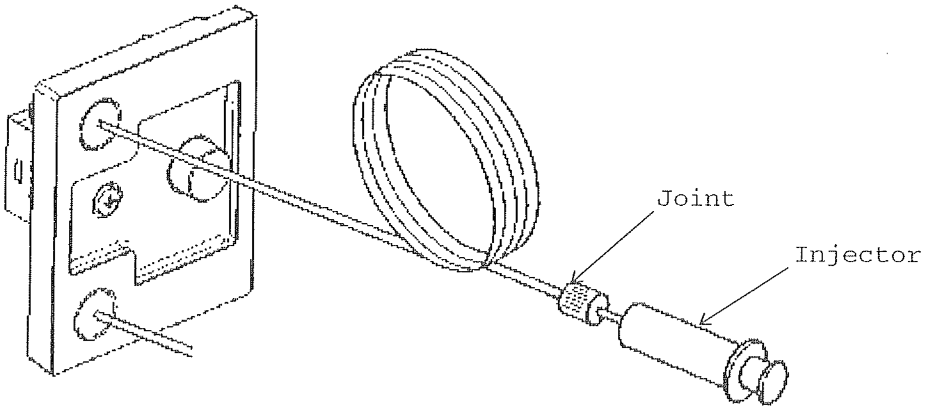
(5) If any contamination is observed inside the cell, it must be cleaned with an appropriate solvent.
Connect the injector to the tip of drain tube, and inject the washing solvent into the cell for cleaning.
If an aqueous mobile phase is used, water should be used.
If an organic based mobile phase is used, ethanol or acetone should be used.

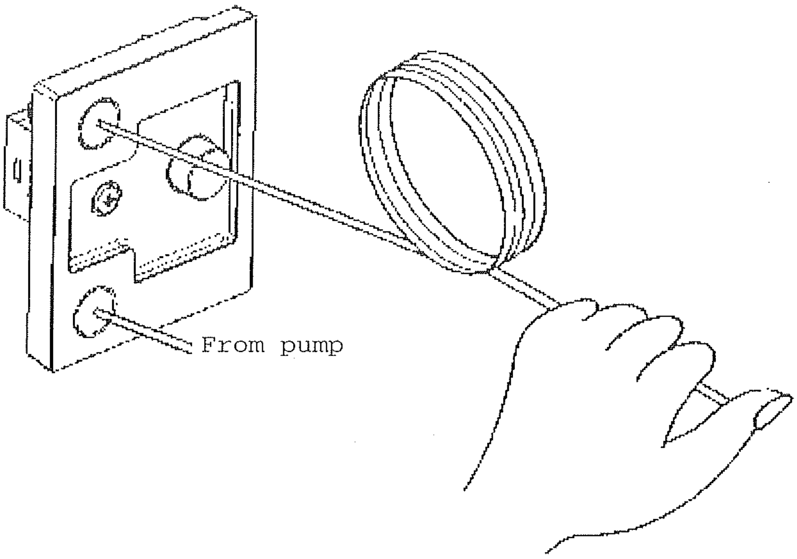
(6) Replace the washing solvent with the eluent that is used for the separation and repeat the process indicated in step (5).

(7) If air bubbles are observed in the cell, deliver mobile phase into the flow cell with the pump using a flow rate of less than 1.0 mL/min and a pressure of less than 1.0 MPa. While the liquid is being delivered to the flow cell, pinch the end of the drain tube with your fingers for a few seconds to increase the pressure inside the cell and release your fingers quickly. Repeat this procedure three or four times until all air bubbles have been removed.

6-5 Checking and Cleaning the Flow Cell Unit


C000013
Flow cell unit
Clamp screw


Joint
Injector



NOTICE: If pressure is too high, the flow cell may be damaged or broken. When removing air bubbles, set the pump pressure limit to 1.0 MPa or less.

6-5-2 Disassembly and Cleaning of Flow Cell

6-5-2-1 Disassembly of Flow Cell

If the contamination in the flow cell cannot be removed by the aforementioned cleaning procedure, disassemble the cell and clean its parts as instructed below.

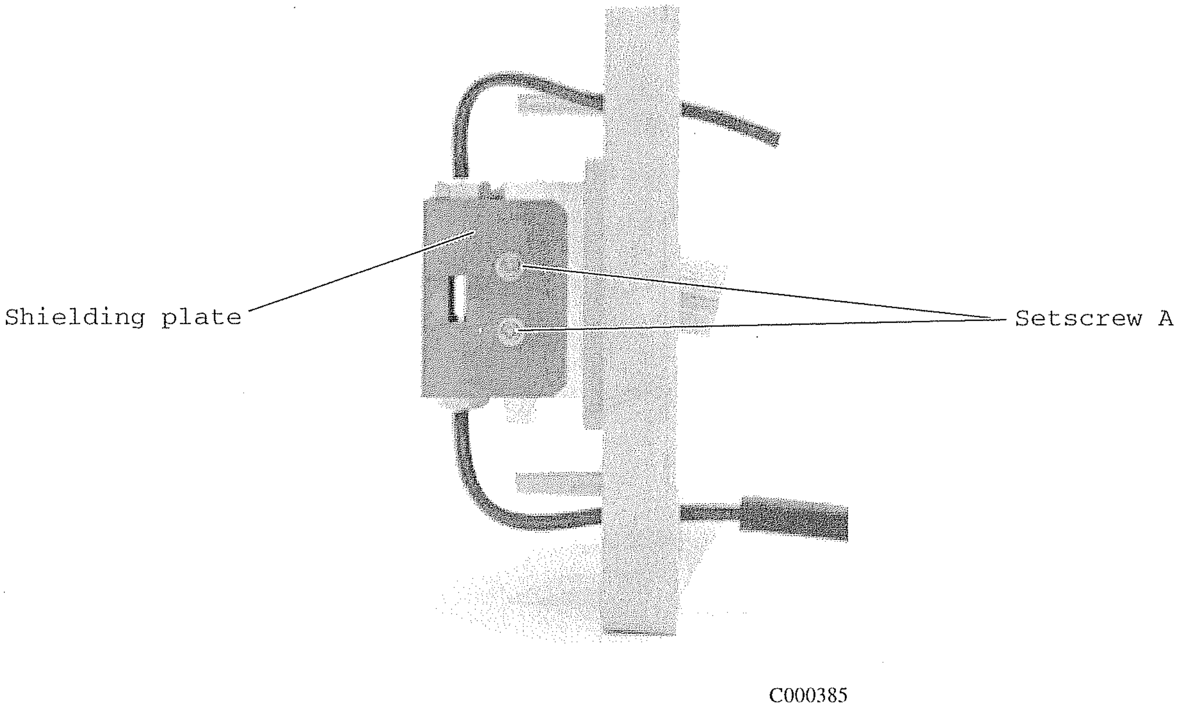


Fig. 6-12 Flow Cell Unit

(1) Loosen setscrews A and detach the shielding plate.

(2) Detach the cell window.

(3) Loosen the upper and lower retaining screws 1 and 2.

(4) Detach the cell cramp.

(5) Take out the flow cell.

6-5-2-2 Cleaning of Flow Cell

(1) Clean the flow cell in running water.
If the contamination cannot be removed with running water, then clean it with an ultrasonic cleaner. In this case be sure to put the flow cell in a paper or plastic container and then place it in the ultrasonic cleaner, so as to avoid scratching the cell.

(2) Wipe the surface of the flow cell with gauze that is moistened with acetone or ethanol.

NOTICE 1: Be careful not to scratch the flow cell surface when disassembling it.

NOTICE 2: Be sure to put the flow cell in a paper or plastic container when cleaning it. Using a glass container may scratch the cell or prevent obtaining the desired performance from the instrument.

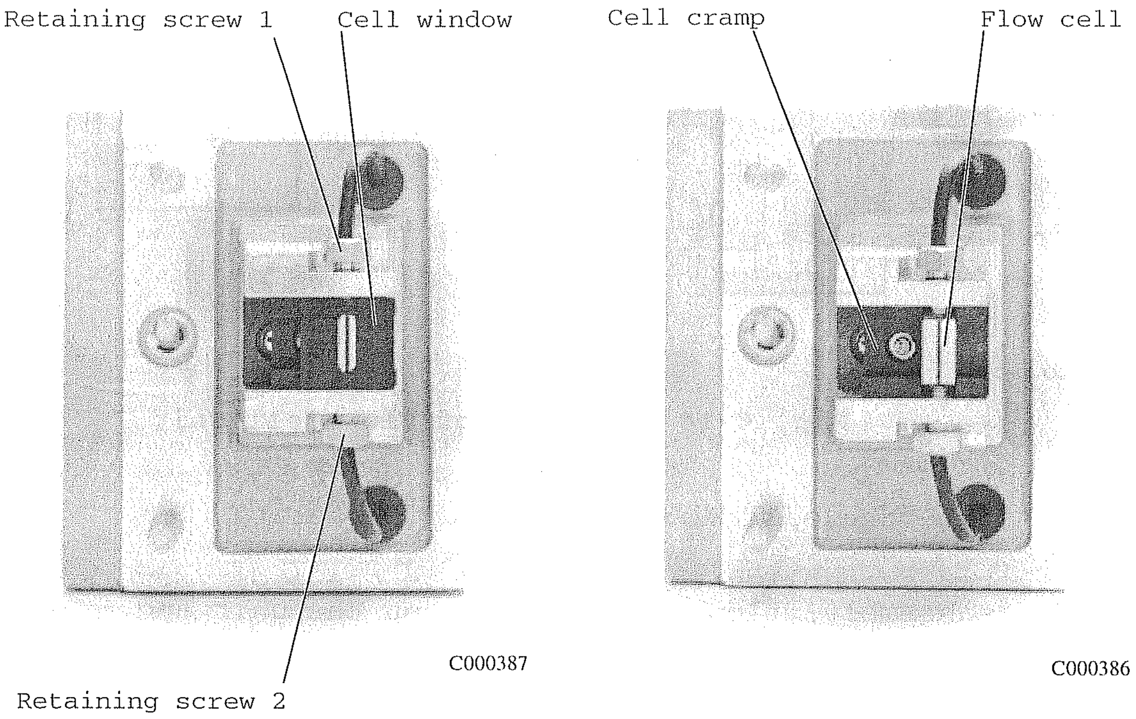
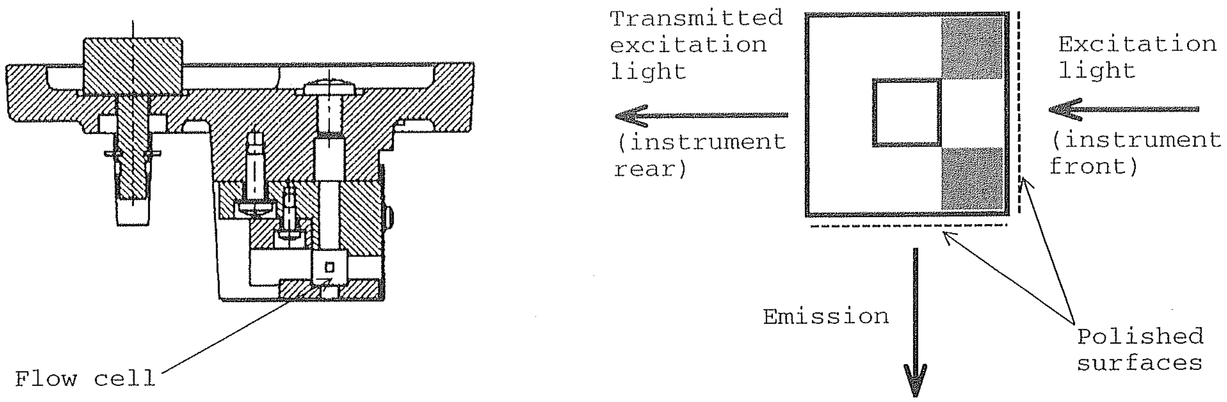

Retaining screw 1
Cell window
Cell cramp
Flow cell
C000387
C000386
Retaining screw 2

Fig. 6-13 Disassembly of Flow Cell Unit

6-5-2-3 Assembly of Flow Cell

(1) Assemble the flow cell into the cell holder in the orientation shown in Fig. 6-14 and fix it with the cell cramp.

(2) Tighten the upper and lower retaining screws.


[View from top]

[Enlarged view of flow cell]

Flow cell
Transmitted excitation light
(instrument rear)
Excitation light
(instrument front)
Emission
Polished surfaces

Fig. 6-14 Assembly of Flow Cell Unit

(3) Attach the cell window and shielding plate.

(4) After assembling the flow cell in the order reverse to disassembly, flow a liquid through the unit and make sure there is no leakage.

NOTICE 1:	Be careful not to scratch the flow cell surface when assembling it.
NOTICE 2:	Take care about the orientation of the polished surfaces of the flow cell when putting it into the cell holder. (See Fig. 6-14.) A mistake in the orientation may preclude obtaining the proper performance from the instrument.
NOTICE 3:	Finger-tighten retaining screws 1 and 2 fully, then tighten another 30° or so with a double-end wrench.

6-5-3 Leakage of the Flow Cell

It is important to check that the flow cell does not leak before collecting analytical data. This check can be performed by monitoring the flow for a few seconds. If the cell leaks, stop the pump immediately and remove the flow cell unit. Use the following procedure to remedy the leak:

(1) Using the pump, feed liquid to check if the flow through the cell is normal (i.e. does the fluid exit the cell and the drain tube to the waste receptacle).

(2) If liquid does not come out of the outlet tube, the tube is clogged. Replace the outlet tube with a new one.

(3) If leakage occurs and liquid comes out of the outlet tube normally, it is possible that the upper nipple may not be securely tightened. Ensure that the upper nipple is finger tight and then secure it using the wrench that is provided (turn it through approximately 10° to 20°).

(4) Remove all liquid on the outside of the flow cell and inside the flow cell compartment.

(5) Before replacing the flow cell, pump the mobile phase to make sure that the problem has been remedied.

NOTICE 1: It is important that the outlet tube of the flow cell is checked first. If the upper fitting is found to be loose and it is retightened with the wrench without checking the outlet tube, the flow cell may be damaged or broken when the fluid is pumped through the cell.

NOTICE 2: If leakage is found in the sample chamber or on the optical base in it, remove liquid thoroughly and clean with a cloth moistened with water.

If liquid comes out of the drain port located at the lower right of the instrument during measurement, stop the pump immediately and turn off the POWER switch. Then, follow steps (1) to (5).

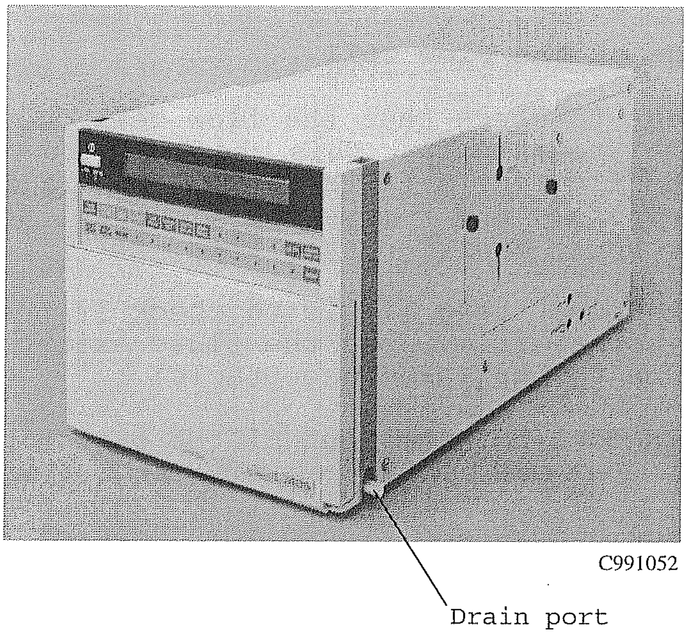


Fig. 6-15 Drain Port

6-5-4 Caution on Storage of Flow Cell

If the flow cell will be left unused for more than a few days, clean the flow cell thoroughly by pumping distilled water or alcohol through it and then store it in a safe place. If the period is less than a few days, the inside of the cell may be filled completely with a solvent like ethanol or acetonitrile and then stored.

6-6 How to Replace the Fuse

⚠ WARNING

Beware of Electric Shock!

Before replacing the fuse, be sure to turn OFF the power switch and unplug the power cord from the outlet.

(1) Obtain a spare fuse (P/N J821349).

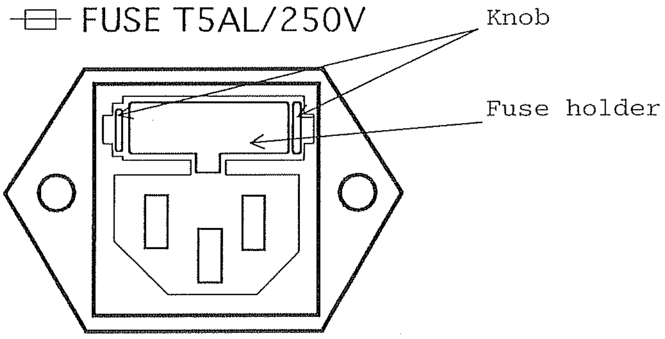
(2) Make sure that the POWER switch of this instrument is turned off, and that the POWER cord is unplugged from the instrument.

(3) Replace the fuse as follows.

   (a) Turn off the POWER switch.

   (b) Unplug the POWER cord from the POWER socket on the rear panel.

   (c) Remove the fuse holder by holding both knobs of its case.



   (d) Replace the existing fuse with a new one, and then push in the fuse holder securely.

6-7 Troubleshooting

NOTICE: If the instrument becomes faulty, contact the dealer from whom you purchased the instrument or the nearest Hitachi service agent. Repair must be left to service personnel who have undergone Hitachi's technical training since there are potential hazards.

⚠ WARNING

Beware of Electric Shock!

Before removing the instrument cover for replacement or adjustment of inside parts, be sure to turn OFF the power switch and unplug the power cord from the outlet.

⚠ WARNING

Touching Hot Part Could Result in Burns!

The light source part (lamp mounting part) becomes extremely hot during operation. Never touch it with hands to prevent burns.
The xenon lamp remains hot for a while even after power-off and can severely burn you if touched.
Although the xenon lamp will be automatically extinguished by a safety mechanism when the light source cover is opened, before replacement of the lamp, turn power off and wait for about 2 hours until it cools down sufficiently.

⚠ CAUTION

Directly Gazing at Illuminated Xenon Lamp Could Cause Eye Damage!

The xenon lamp radiates intense ultraviolet light when it is on.
Do not look at the xenon lamp directly when it is lit. Be sure to wear tinted safety glasses to prevent possible eye damage.

Symptom	Cause	Judgment/Check	Remedy
1. Self-diagnosis (initialization) is not performed when the POWER switch is turned on.	The power cord plug is not securely plugged in.	Visual check	Plug in the power cord securely.
	The fuse is blown.	Check the fuse for continuity.	Replace the fuse with a new one. (Refer to Section 6-6.)
	The lamp cover is not properly attached. (The cover activates a safety interlock switch.)	Visual check	Retighten the retaining screws of the lamp cover.
2. The keyboard does not work.	The instrument is not ready for operation.	Check the monitor screen.	Press the ESCAPE key. The READY status will be presented.
3. The AUTO/ZERO or SPECT/MEMO key does not work.	On the monitor screen, the data mode is not set to FL.	Check the monitor screen.	On the monitor screen, set the data mode to FL. (Refer to Section 3-3 (8).)
4. The time program does not start.	The instrument is not ready to operate.	Check the monitor screen.	Press the ESCAPE key. The READY status will be presented.
5. Parameters are not printed out on the D-7500 when the PRINT function is turned using the UTILITY key.	The D-7500 is not ready.	Check the status of the D-7500.	Set the D-7500 to the READY status.
	The D-line communication link is not turned on.	On the monitor screen, check the D-line communication status.	Turn on the D-line communication through SETUP.
	The D-line cable is not connected properly.	Visual check	Check to ensure that the D-line cable is connected.

(cont'd)

Symptom	Cause	Judgment/Check	Remedy
6. Excessive noise	The flow cell is contaminated.	Visual check	Clean the flow cell. (Refer to Section 6-5-1.)
	The mobile phase is not sufficiently degassed.	A spike occurs.	Degas the mobile phase thoroughly.
	The mobile phase contains impurities.	Check the mobile phase.	Purify the mobile phase, or replace it with mobile phase of a higher quality.
	The gain level of the recorder is too high.	Check the gain level of the recorder.	Adjust the gain level of the recorder.
	The pump is faulty.	Check to ensure that the operation of the pump is smooth.	Check the pump.
		Check for noise when the pump is stopped.	
	The xenon lamp has reached the end of its useful life.	The S/N is less than 120/1.	Replace the xenon lamp with a new one. (Refer to Section 6-2.)
7. Excessive drift	The warm-up period is not sufficient.	—	Wait until the instrument becomes stable (at least ten minutes).
	Mobile phase is leaking from a fitting.	Check each fitting.	Retighten the offending fitting.
	An impurity (a strongly retained compound) is eluting from the column.	Stop feeding liquid from the pump.	Wait until elution is completed, or replace the column with a new one.
	There is a significant change in the ambient temperature.	—	Control the temperature variation.
	The mobile phase is not sufficiently pure.	Check the mobile phase solvent.	Purify the mobile phase, or replace it with one of a higher quality.

(cont'd)

Symptom	**Cause**	**Judgment/Check**	**Remedy**
8. Poor S/N ratio	Flow cell contaminated	Visual check	Clean the flow cell (see 6-5-1).
	Xenon lamp off-position	Check lamp energy level	Adjust lamp position (6-1-2).
	Error in flow cell mounting	Visual check	Correctly mount flow cell (6-5-2).
	Flow cell surface scratched	Visual check	Replace flow cell (6-5-2).

6-8 Error Messages

Message	Description	Remedy
D-LINE SYSTEM IS NOT READY	The communication parameter setting or the D-line cable connection is not set properly. Some other device connected through the D-line cable (e.g. the Integrator) is not ready for communication.	(1) On the monitor screen, check that the D-line condition is properly set. (2) Check that the D-line cable is properly connected. (3) Check the status of the device connected through the D-line cable.
A/Z OVER RANGE PRESS "CL" KEY TO CLEAR MESSAGE	The allowable auto zero range is exceeded.	(1) Change the wavelength setting. (2) Use a mobile phase with a lower level of fluorescing impurities. (3) Clean the flow cell.
LAMP ERROR	The lamp goes off during analysis, the lamp does not turn on, or a momentary power interruption is encountered.	(1) Press the CL key to reset the system (after a momentary power interruption). (2) If the lamp does not start, turn the power off and turn it on again. (3) If the lamp is at the end of its useful life time, replace it with a new one.
Ex(Em) SIDE OF WL DRIVE MECHANISM ERROR	The monochromator cannot properly set during the self-diagnosis test (initialization).	(1) Turn power on again. (2) Notify the nearest Hitachi service center.
ROM ERROR	Error in ROM	Notify the nearest Hitachi service center.
C-RAM ERROR	Error in RAM	Notify the nearest Hitachi service center.
CHECK TIME PROGRAM	The allowable maximum number of time program steps is exceeded.	Remove unnecessary steps from the time program.
OVER HEAT! LAMP WAS TURNED OFF	The fan has stopped and the temperature sensor was activated.	The fan is faulty. Notify the nearest Hitachi service center.
	The lamp becomes too hot (it is not cooled down by the fan after turn-off.)	After the lamp cools down, turn on power on again.

(cont'd)

Message	Description	Remedy
D-LINE COMMUNICATION ERROR PRESS "CL" KEY TO CLEAR MESSAGE	Some other device connected via the D-line cable (e.g. the Integrator) is not ready for communication.	(1) Check connection of the D-line cable. (2) Check the status of the device connected through the D-line cable.
Ex ENERGY Ex ACCURACY Em ACCURACY oooo NOT DETECTED CALIB. PEAK	In the wavelength check mode, a peak cannot be detected properly.	(1) Check that the filter is set correctly. (2) Check that the flow cell is filled with water. (3) Check that there is no significant deviation in the numeric value setting for wavelength accuracy calibration.
LAMP WAS TURNED OFF BY LAMP COVER OPEN TURN OFF THE POWER	Lamp cover is open.	(1) Close the lamp cover. (2) Turn on power supply again.
NO SPECTRA OR SPECTRA ARE MISMATCHED PRESS "CL" KEY TO CLEAR MESSAGE	No spectral data, scan mode in-correct, fixed wavelength incorrect, background WL range doesn't include entire spectrum WL range, or background slit position differs from spectrum slit position.	Select correct parameters.
PARAMETER ERROR	Error in parameter data (data cannot be retained due to EEPROM error)	(1) Turn on power again. (2) Contact your nearest Hitachi service center.

NOTICE: An input value stored in this instrument may be deleted if certain error messages are presented. If this is the case, enter the relevant value again. In the event of PARAMETER ERROR, the wavelength calibration value is deleted (see Section 6-4-3). In this case, it is necessary to perform the wavelength calibration procedure (see Section 6-4).

7. REPLACEMENT PARTS AND CONSUMABLES

Table 7-1 lists the replacement parts and consumables for this instrument. It is suggested that a suitable quantity of each item be stocked in the laboratoty to minimize down time. For many of these items (e.g. lamps), the quantity is dependent on the level of use of the instrument.

Table 7-1 Replacement Parts and Consumables

Part Number	Part Name	Remarks
*650-1500	150 W xenon lamp	Guaranteed service life: 150 hours or 6 months
*250-1600	Long-life xenon lamp (option)	Guaranteed service life: 500 hours or 6 months
J821349	Fuse T 5 A	Guaranteed service life: 1920 hours/year
810-3927	Flow cell (12 μL)	Only cell
810-3859	Nitoflon tube ID 0.33 × 0.62 T (outlet)	Length: 2 m Outside diameter: 1.57 mm
810-3853	Nitoflon tube ID 0.25 × 0.66 T (inlet)	Length: 1 m Outside diameter: 1.57 mm

* Indicates a consumable item.

NOTICE: The intensity of an unused Xe lamp that has been stored for a long period of time (i.e. a year) will be less than that of a newly acquired lamp. It is suggested that the user maintain a small supply of spare lamps and reorder lamps as required, rather than purchasing a large number of lamps at one time.

8. DESCRIPTION OF CONTACT COMMUNICATION

8-1 Outline

Two types of contact signal are used in the L-7000 series.

(1) Contact Signals (START, STOP, BUSY) Entering the D-line Connector

These are contact signals which have input/output functions that are used to communicate between each unit.
These signals are established via the START (IN/OUT), STOP (IN/OUT) and BUSY (IN/OUT) lines and are valid when the D-line is turned OFF via the setup function of each unit.

(2) Individual Contact Signals Used by Each Unit

These are contact signals that are input and output via 3-pin connectors. Their names and functions vary with the unit. A name that indicates the function is given at each connector.

Example of output contact signal : EVENT (pump)
Example of input contact signal : LAMP OFF IN (detector)
SERIES START IN (autosampler)

8-2 Contact Signals Entering the D-line Connector

Each unit has two D-line connectors at the rear of the main unit (except for the data processor which has one). By connecting each unit in series via the D-line cable, synchronization of the activity of all units in the L-7000 series system is achieved (e.g. a single start command initiates the activity of all units in the system.). An example of D-line cabling is shown in Fig. 8-1.

To synchronize operation with the L-6000 series and/or D-2000/2500 or D-6100/6500 by way of contact signals, use the cables indicated below to make the connection. For connection with the instrument bearing the CE conformity marking, the part numbers indicated on the second line are applicable.

(1) For Synchronized Start Only

Part No.	Part Name	Opposing Terminal	Opposing Device (example)
810-7634 810-7514	SDIO(START 3P) cable FDIO(START 3P) cable	3P connector	L-6200, D-6100
810-7633 810-7513	SDIO(START M3) cable FDIO(START M3) cable	M3 terminal	L-5000
810-7632 810-7512	SDIO(START M4) cable FDIO(START M4) cable	M4 terminal	D-2500/2000

(2) For Synchronized Start, Ready/busy and Stop

Part No.	Part Name	Opposing Terminal	Opposing Device (example)
810-7631 810-7511	SDIO cable FDIO cable	3P connector	L-6200, D-6100

If the opposing terminal is not a 3P connector, it is necessary to connect a separate cable to the end of the SDIO cable.

NOTICE 1: Be sure to use the cables indicated for connection of the contact signals.

NOTICE 2: To synchronize systems that include units that are not part of the L-7000 series by way of contact signals, use the relay box (P/N 810-7630).

NOTICE 3: The contact terminals have polarity. Be certain to connect cables using the correct polarity.

NOTICE 4: The voltage applied across contact terminals must not exceed 30 V.

NOTICE 5: When shorting across contact terminals, a maximum current of 10 mA will flow for each unit in the system. If multiple units are connected via the D-line cable, this current will be additive (e.g. 3 units = 30 mA). Make certain that contacts which can withstand a current of at least 0.1 A are used for the contact start circuit on the opposing side.

NOTICE 6: The maximum current of the contact output (start) circuit is 0.1 A. The contact signal reception circuit on the opposing side should be checked to ensure that this current will not cause any damage before the unit is connected to the D-line system.

NOTICE 7: A diagram of the D-line contact circuit is presented in Fig. 8-2.

8-3 Individual Contacts to Each Unit

(1) Contact Input Terminals

Contact signal input terminals are used for control using contact signals from an external switch, relay, etc.
A signal is activated by shorting the terminal for a minimum of 0.1 seconds. The input circuit is shown in Fig. 8-3.

(2) Contact Signal Output Terminals

Contact signal output terminals are used for control using contact signals from an external unit. The rating of the contacts is 30 V, 0.1 A and care should be taken to ensure that the rating is not exceeded. The output circuit is shown in Fig. 8-4.

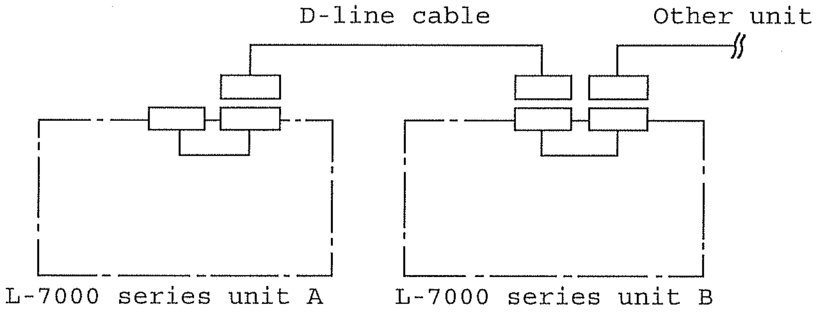

D-line cable
Other unit
L-7000 series unit A
L-7000 series unit B

Fig. 8-1 D-line Cable Connection

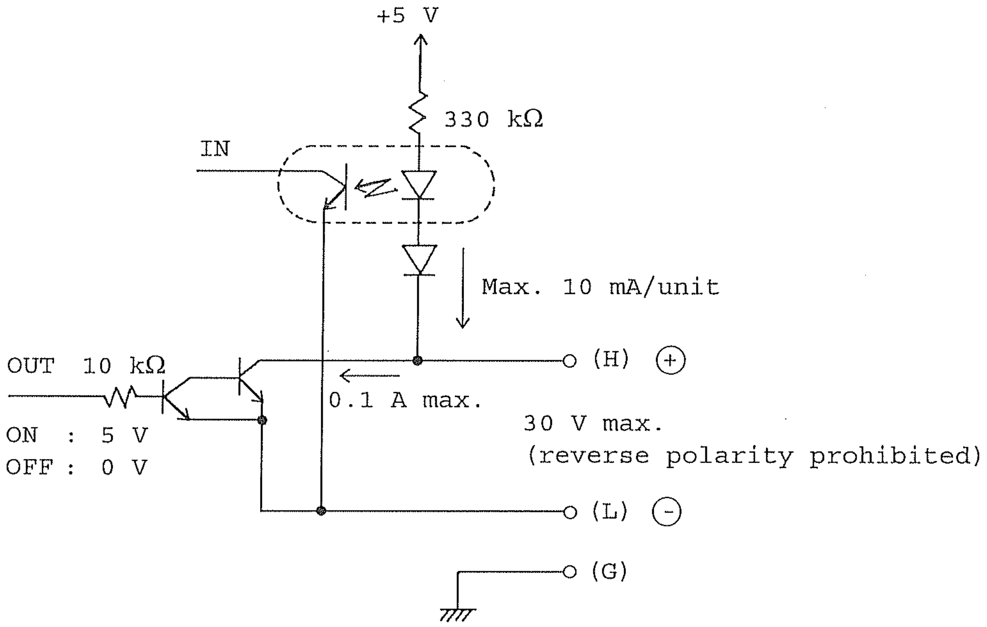

+5 V
330 kΩ
IN
Max. 10 mA/unit
OUT 10 kΩ
(H) +
0.1 A max.
ON : 5 V
OFF : 0 V
30 V max.
(reverse polarity prohibited)
(L) −
(G)

Fig. 8-2 D-line Contact Circuit Configuration

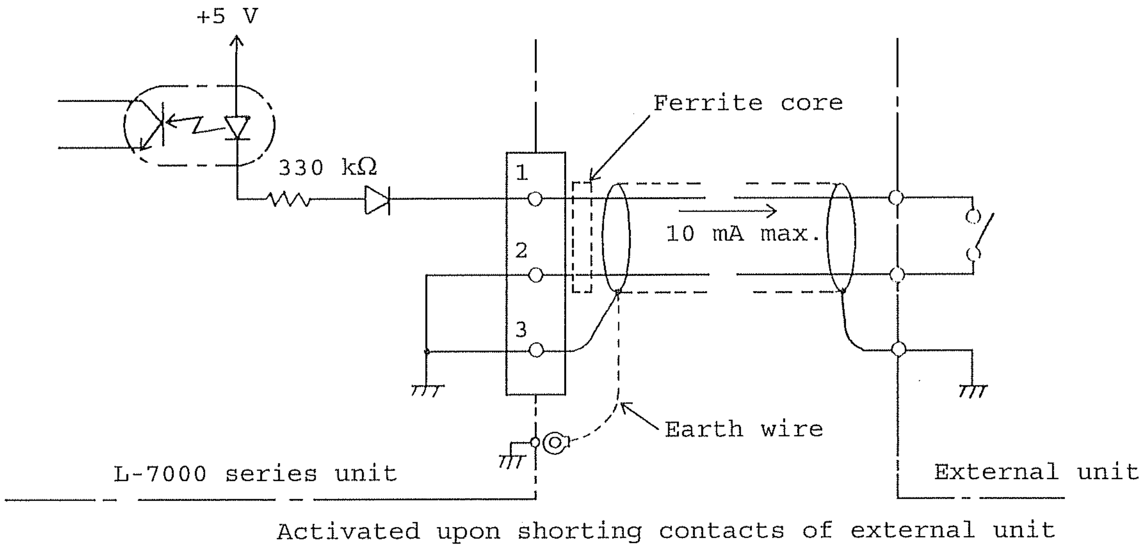

+5 V
330 kΩ
Ferrite core
1
2
3
10 mA max.
Earth wire
L-7000 series unit
External unit
Activated upon shorting contacts of external unit

Fig. 8-3 Contact Signal Input Circuit

NOTICE: For connection with the instrument bearing the CE conformity marking, use the dedicated cable having ferrite core and connect the ground wire to the ground terminal ⏚ .

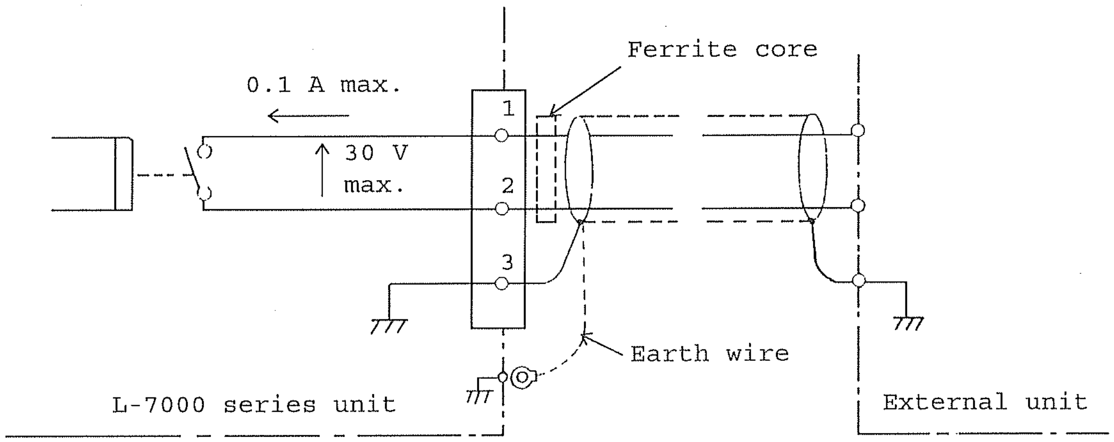

Ferrite core
0.1 A max.
30 V max.
1
2
3
Earth wire
L-7000 series unit
External unit

Fig. 8-4 Contact Signal Output Circuit
(There is no output terminal on Model L-7485)

NOTICE: For connection with the instrument bearing the CE conformity marking, use the dedicated cable having ferrite core and connect the ground wire to the ground terminal ⏚ .

APPENDIX

A. Terminology

Auto zero	The intensity of fluorescence is adjusted to zero electrically to compensate for unnecessary data. In a common analytical practice, perform auto-zero immediately before or after analytical operation.
Auto zero in	The external contact signal can be input for auto-zero adjustment.
Battery backup	This function is incorporated to retain measurement parameters and other analytical conditions even after power is turned off.
Confidence	This function is used for confirmation of instrument operating status.
Diffusion plate	This quartz plate is used for diffusion of excitation beam.
D-line	Digital network dedicated for analytical operation.
Drain port	Equipped for allowing solution leaked from the flow cell/joint to an external waste bottle.
Excitation wavelength	Used for exciting a sample.
Flow cell	Used for detection in fluorescence measurement.
Fluorescence	A kind of luminescence, which is emitted from a sample when a stimulating excitation beam is given to it.
Fluorescence intensity	Unit of measure of fluorescence (relative intensity).
Fluorescence wavelength	A particular wavelength of fluorescence emission caused by excitation.
Fullscale	Used to define a magnitude of output to be delivered to such an external device as data processor (integrator).

Initial screen	This screen appears at startup of the instrument.
Key lock	This function disables key operation to protect against an erroneous input during operation.
Lamp energy	Represents the intensity of lamp radiation. This value is used as an approximate value of lamp service life.
Lamp holder	Equipped for holding the light source lamp. This part becomes extremely hot when the lamp is lit. Be careful not to cause burns by touching it.
Lamp-off in	The external contact signal can be input for turning off the light source lamp.
Lamp turn-on count	Indicates the number of times the light source lamp has been turned on.
Lamp turn-on cumulative time	Indicates a cumulative total time of lamp turn-on periods.
Log book	Means the information containing records of instrument operating conditions.
Marker in	The external contact signal can be input to provide marking on record.
Offset	On output, a preset input signal value is added to an actually measured value. This function is particularly useful when the baseline varies on the negative side.
Output range	Used to define a magnitude of output signal to such an external device as data processor (integrator).
Photomultiplier	Used to detect fluorescence emission.
PROCESSOR terminal	Used for analog output to the data processor (integrator).
Raman scattering	In Raman effect, a kind of scattered light appears on the side of wavelength slightly longer than excitation wavelength.
Raman spectrum	Scattered light spectrum that occurs due to Raman effect.
RECORDER terminal	Used for analog output to a recorder.
Scan	Means a sequential shifting operation of monochromator wavelength for spectral measurement.

Scattered light	Includes Raman scattered light, 2nd-order light, 3rd-order light, etc. In fluorescence analysis, particular attention should be paid to scattered light.
2nd-order light	A spectral peak (a kind of scattered light) which appears at a wavelength of 2 times the level of excitation intensity. In a common arrangement, the filter is used to remove or cut off the 2nd-order scattered light.
Spectra memory	This function stores fluorescence or excitation spectral data of samples.
Stokes law	This law states that the wavelength of a fluorescent emission is always longer than that of excitation wavelength.
3rd-order light	A spectral peak (a kind of scattered light) which appears at a wavelength of 3 times the level of excitation intensity. In a common arrangement, the filter is used to remove or cut off the 3rd-order scattered light.
Time program	Used to carry out measurement at different measurement (excitation/fluorescence) wavelengths according to the point of time (analyte component).
Xenon lamp	The xenon lamp is used for fluorescence measurement.

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Standard Operating Procedure SOP

Title: Check of Function and Specifications of the Fluorescence Detector L-7485

Valid since: June. 1. 2000
This SOP replaces the version of: New version, issue 1

Policy: Establishing a reliable function of the instruments
Responsibility: Head of Laboratory

Data/signature Copy received by:

Written: .... Head of Testing Facility
.... Head of Laboratory
.... Laboratory personnel
.... GLP department
Reviewed: ....
Approved: ....

Total number of pages: 10 and 1 page Test Report
References: Operating Instructions for Fluorescence Detector L-7485.
ASTM E685-79 (1979)

Check Item: Four Items

1 Check of lamp display
2 Check of auto zero function
3 Check of wavelength accuracy
4 Check of sensitivity

Items to be Prepared:

1. Ion exchanged water or distilled water: About 100 mL.
2. Calipers.
3. Calibrated data processor for HPLC (Hitachi Model D-7500 Integrator or equivalent), or recorder (Hitachi Model 056 Recorder or equivalent).
4. Pump for HPLC (Hitachi Model L-7100 Pump or equivalent).
5. Analog signal cable (necessary when a data processor for HPLC except for Model D-7500 or a recorder is used).

Preparation of Instruments:

1. Turn on the Fluorescence Detector and warm it up for about 1 hour.
2. Turn on the data processor for HPLC and recorder. Warm them up for about 10 minutes.

1 Check of Lamp Display

Content of Check:

Lamp display check.

Check Procedure:

1. Turn on the Power switch of the Detector.
2. The screen appears on the liquid crystal display.
3. Self-diagnosis is performed.
   If abnormal, Error message is presented on the liquid crystal display. If normal, Monitor Screen appears.

Actual Example:

POWER

```
FL Detector    8107064-00
Copyright (C)   Hitachi, Ltd.   2000
```

NOTICE: Software version may be changed.

↓ (Self-diagnosis check)

```
TIME    Ex    Em     FL    PROG    LAMP   [1]
0.0     250   350    0.0      1      Xe   (D1)
```

NOTICE: This screen is an example.

Specification:

Lamp display on Monitor Screen should be {Xe}.
This means that the Xe lamp is lit normally.

Remarks:

The following items are checked by the self-diagnosis check.

1. Memory check.
2. Function check of wavelength motor.
3. Lighting check of Xe lamp.

2 Check of Auto Zero Function

Content of Check:

Auto zero function is checked.

Check Procedure:

1. Make sure Monitor Screen (FL display) is displayed.
2. Set the excitation wavelength to 350 nm, and fluorescence wavelength to 450 nm with the WAVE LENGTH key.
3. Set the time constant to 2.0s with the PARAM SET key.
4. Press the AUTO ZERO key and read the FL display on Monitor Screen.

Specification:

FL display on Monitor Screen should be -0.1 to +0.1.

NOTICE: When a solution that emits fluorescence of which wavelength is 450 nm with the excitation light of 350 nm is in the cell, the FL display may be out of ±0.1 in some cases. If so, substitute distilled water for the solution in the cell, and perform measurements again.

3 Check of Wavelength Accuracy

Content of Check:

The spectral line of 436 nm emitted from a fluorescent lamp is used.
The light emitted from a fluorescent lamp is introduced into the fluorescence monochromator and the spectral line is used to check wavelength accuracy of the monochromator.
Using the emission monochromator set at the wavelength at which the bright line is detected, the wavelength accuracy of excitation monochromator is determined by checking the wavelength of excitation light which makes Raman scatter at 436 nm and comparing with theoretical value(380 nm).

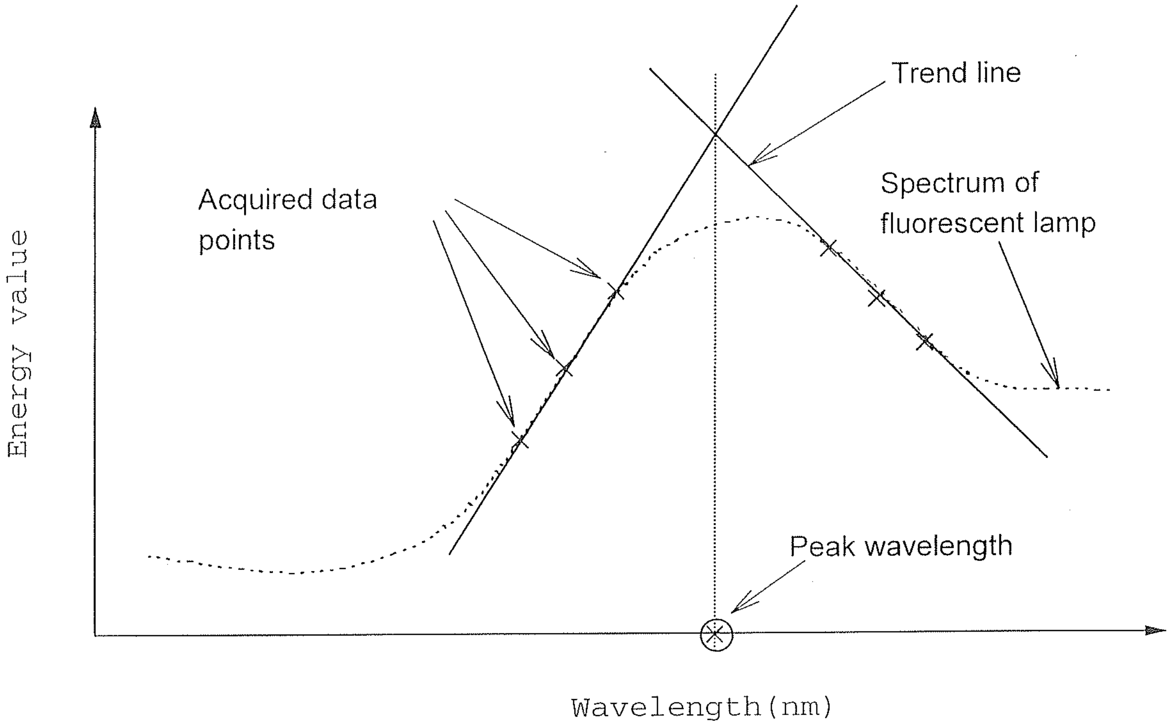
Items to be Prepared:

1. Fluorescent room lamp
2. Graph sheet

Check Procedure:

1. Check of wavelength accuracy of fluorescence monochromator

1a. Remove the light sampling screw of the flow cell unit.
1b. Set PMT voltage "LOW" with the PARAM SET key.
1c. Set Em bandwidth "STANDARD" with the PARAM SET key.
1d. Turn off the lamp with the PARAM SET key.
1e. Make the Display Mode be Em < M > with the UTILITY key.
1f. Set the fluorescence wavelength (Em) to 426 nm with the WAVE LENGTH key, and read the energy value.
1g. In the same way, read the energy values at 428nm and 430nm.
1h. Plot these points on a graph sheet and confirm them in a row. If not, add another data point and acquire at least three data points in a row.
1i. Draw a trend line of these points.
1j. In the same way as (f) and (g), read the energy values at 442nm, 444nm and 446nm.
1k. Draw another trend line of these points in the same way as (h) and (i).
1l. Read the wavelength at the cross point of the two trend lines.


Trend line
Acquired data points
Spectrum of fluorescent lamp
Energy value
Peak wavelength
Wavelength(nm)

NOTICE 1:	When checking the accuracy of the fluorescence monochromator, take special precautions to ensure that radiation from other sources cannot enter the instrument (e.g. do not place the unit near a window or near a source of room light).
NOTICE 2:	If you move around in front of the sample chamber during the check of the fluorescence wavelength accuracy, the observed intensity of the fluorescent lamp light may vary. This may prevent you from accurately determining the peak wavelength.
NOTICE 3:	If the energy level appears to be saturated during the determination of the wavelength accuracy, adjust the amount of light entering the monochromator by turning off some lamps in the laboratory.
NOTICE 4:	In wavelength accuracy check, set the emission monochromator bandwidth at the standard (15 nm).

2. Check of wavelength accuracy of excitation monochromator
2a. Attach the light sampling screw of the flow cell unit.
2b. Set the fluorescence wavelength to the position determined in the procedure of 1l. above.
2c. Turn on the lamp with the PARAM SET key.
2d. Set PMT voltage “MED” with the PARAM SET key.
2e. Make the Display Mode be Em < M > with the UTILITY key.
2f. Set the wavelength of the excitation monochromator (Ex) at 380 nm with the WAVE LENGTH key.
2g. Move the wavelength of the excitation monochromator (Ex) with the WAVE LENGTH key to obtain the wavelength where the displayed energy becomes its maximum.

NOTICE: Adjust the Xe lamp position properly, then check the wavelength accuracy of the excitation monochromator.

Specification:

Obtained wavelength should be;
Ex: within ±3 nm of 380 nm
Em: within ±3 nm of 436 nm

4 Check of Sensitivity

Content of Check:

Sensitivity of Fluorescence Detector is measured by measuring S/N value using Raman Spectrum of water.

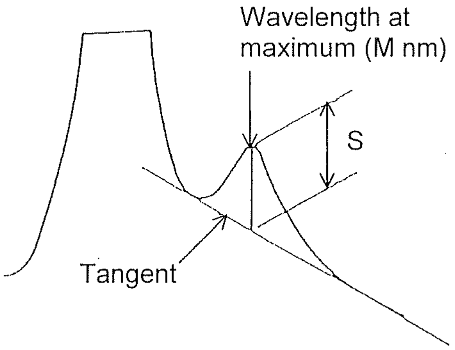
Items to be Prepared:

1. Prepare the following.
1a. A calibrated data processor for HPLC (Hitachi Model D-7500 Integrator or equivalent) or a recorder (Hitachi Model 056 Recorder or equivalent).
1b. Calipers.
1c. Load resistance coil (Teflon tube, inside diameter: 0.25 mm, length: 10 m).
2. Adjust the lamp position.
3. Flow ion exchanged water or distilled water with a flow rate of 1 mL/min for 3 minutes. Make the flow rate be 0 to stop the flow. Close the inlet and outlet of the Flow Cell tube. When flowing, connect a load resistance coil before the Flow Cell.
4. Connect the data processor for HPLC and Detector with the Signal Cable. When a recorder is used, connect the Signal Cable to “Recorder” terminals.

Check Procedure:

1. Measurement of Raman spectrum of water
1a. Turn on the Power switch and warm up the instrument for more than 1 hour.
1b. Set the wavelength of the excitation monochromator (Ex) at 350 nm with the WAVE LENGTH key.
1c. Set the recorder output speed at 60 nm/min with the RECORD key.
1d. Set the recorder full scale range at 1000 with the RECORD key.
1e. Set the time constant to 2.0 s, set the applied voltage to the photomultiplier to “Medium” with the PARAM SET key.
1f. Set the scan mode to Em, set the fluorescence spectrum measurement wavelength at 350 nm to 450 nm with the SPECT PARAM key.
1g. Start scanning, and input memory number to be used for storing with the SPECT MEMO key.
1h. Set the spectrum number of output data, and background data with the RECORD key. Output the spectra with the START STOP key.
Start the data processor or recorder at the same time for recording.

1i. Obtain Raman peak intensity (s) and wavelength at the maximum (M nm).



NOTICE 1: Use the Model D-7500 Integrator with the following conditions.
ATT: 5
Chart speed: 60 mm/min
Sampling period: 400 msec
NOTICE 2: When the Detector and Integrator are connected with the D-line (digital), start the output with the ALL START key of the Integrator.

2. Measurement of noise
2a. Keep the excitation wavelength at 350 nm, set the fluorescence wavelength at M nm with the WAVE LENGTH key.
2b. Start the Integrator/Recorder, record for 15 minutes.
2c. From the recorded chart, calculate the noise following ASTM E685-79 (1979).

NOTICE: Use the Model D-7500 Integrator with the following conditions:
ATT: 0
Chart speed: 10 mm/min
Sampling period: 400 msec

3. Calculation of S/N value (sensitivity) of Raman spectrum of water

$$\text{S/N (value)} = \frac{S}{N}$$

Specification:

200 or more

NOTICE: Quality of distilled water and bubbles in water affect Raman spectra of water and noise level.
Select high quality water with no bubbles.
Incomplete adjustment of the optical system, a dirty optical system or Flow Cell may reduce S/N value.
If lamp replacement is not effective to increase S/N value, contact your nearest Service Office to ask for adjustments.

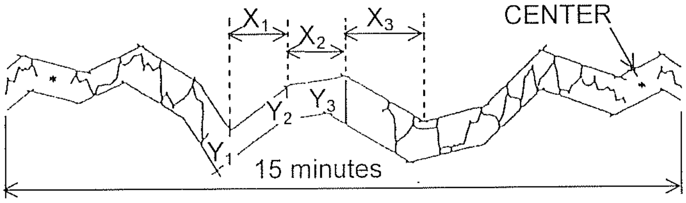
Remarks:

When the Model D-7500 Integrator is used, the voltage value corresponding to "S" can be calculated from the length of S (L mm) on the chart, and the noise level can be calculated from the noise width (mm) on the chart.

$$S = \frac{L}{152} \times 2^{x} (\mathrm{mV})$$

$$N = \frac{\text{Noise width}}{152} \times 2^{x} (\mathrm{mV})$$

Where; L is the length of S (mm)
X is the ATT (attenuator) setting on the Integrator,



Noise level = $\sum_{R=1}^{R=n} Y_R / n$ (Unit of Xn is 1 min)

Where: Y_R is a length (mm) on chart at each time period.
(Extracted from ASTM E685-79 (1979))

Module	**Fluorescence Detector**
Model	**L-7485**
Instrument number	

Test

No.	**Item Tested**	**Specification**	**Result**	**Tester**
1	Lamp display	{Xe} should be displayed		
2	Auto zero function	Within ±0.1		
3	Wavelength accuracy ① Excitation wavelength ② Fluorescence wavelength	 Within ±3 nm Within ±3 nm		
4	Sensitivity	S/N of Raman peak of water should be 200 or more		

Tested on: ....

Next test scheduled for: ....

Tested by: ....